Cape Peninsula University of Technology

EVALUATION OF THE LABELLING AND BINDING EFFICIENCY OF Tc-99m TO RED BLOOD CELLS OF PATIENTS WHO ARE BEING EXPOSED TO A COCKTAIL OF ANTI-TUBERCULOSIS DRUGS

by

CAROLYNN LOUISE LACKAY

Student number 197084834

Thesis submitted in fulfilment of the requirements for the degree

Master of Technology: Radiography (Nuclear Medicine)

in the Faculty of Health Sciences

at the Cape Peninsula University of Technology

Supervisor: **Shafick Hassan**
Co-supervisor: **Sietske Rubow**

Bellville
September 2010

DECLARATION

I, Carolynn Louise Lackay, declare that the contents of this thesis represent my own unaided work, and that the thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

Signed **Date**

ABSTRACT

Introduction: Radioactively labelled red blood cells (RBC) are used in various nuclear medicine studies. In order to obtain accurate results when performing these studies, it is of paramount importance that a good binding of the radioactivity (Tc-99m) with the red blood cells is ensured. The literature indicates that certain drugs can influence red cell membrane properties and biochemistry. These drugs can potentially influence the binding of radionuclides to cells. Antibiotics may possibly alter the labelling efficiency of Tc-99m RBC. Due to the high incidence of tuberculosis (TB) in South Africa, many patients receive anti-TB medication, and therefore the influence of these drugs on the labelling efficiencies of Tc-99m RBC was studied.

Materials and Methods: 45 newly diagnosed patients (males and females aged between 18 and 55 years) with confirmed pulmonary tuberculosis (PTB) were enrolled in the study after informed consent. Blood samples were obtained from the enrolled patients before commencement of anti-TB treatment (t0/baseline study), as well as 1 month after commencement of treatment (during intensive phase of treatment) and 4 months into their treatment (during continuation phase). Treatment consisted of an initial 2 months phase during which patients received rifampicin, isoniazid, pyrazinamide and ethambutol, followed by a 4 month continuation phase of rifampicin and isoniazid only. *In vitro* RBC labelling was performed on each blood sample in duplicate and the labelling efficiencies were determined for each sample. Results were analysed by non-parametric statistical methods (ANOVA).

Results: Data up to 4 months was obtained in 33 out of the 45 enrolled patients. Baseline studies yielded an average labelling efficiency (LE) of 96.5% ± 0.45%. During the mid-point of intensive phase of treatment (1 month) the average LE obtained was 95.2% ± 0.75%. The average LE for the mid-point of the continuation phase of treatment (4 months) was 96.7% ± 0.53%.

Discussion: Labelling efficiencies (LE) with and without anti-TB medication were well above 90%. No statistically significant difference could be found between the LE of the baseline studies and the labellings done during the two phases of treatment.

Conclusion: Anti-tuberculosis drugs do not affect *in vitro* RBC labelling with Tc-99m, and should thus not interfere with nuclear medicine investigations.

ACKNOWLEDGEMENTS

I wish to thank:

- The patients for their participation in the study.
- The volunteers for donating blood samples.
- Professor Sietske Rubow, radiopharmacist in the Nuclear Medicine division at Tygerberg Hospital, for guidance with this project and for her endless encouragement.
- Shafick Hassan (head of department, Health and Wellness Sciences at Cape Peninsula University of Technology) for assistance with the administration of this project.
- The Cape Peninsula University of Technology for financial support.
- The Desmond Tutu TB Centre for assistance with patient recruitment.
- Sr Danita Bester and Sr Susan van Zyl (Desmond Tutu TB Centre) for blood sample collection.
- Professor Daan Nel (Head Statistician, Centre for Statistical Consultation at Stellenbosch University) for assisting with the statistical analysis of the data.
- The staff of the Nuclear Medicine division of Tygerberg Hospital for their support.
- GBM.

DEDICATION

For my brother, Julian.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ADDENDUMS

LIST OF ABBREVIATIONS

ACD-A	:	Acid citrate dextrose-solution formula A
ALA-S	:	Amino-levulinic acid synthetase
Cr-51	:	Chromium-51
E	:	Ethambutol
EPTB	:	Extra-pulmonary tuberculosis
G	:	Gauge
g	:	Gravitational force (g-force)
Ga-67	:	Gallium-67
GIT	:	Gastro-intestinal tract
GSH	:	Glutathione
H or INH	:	Isoniazid
Hb	:	Haemoglobin
HIV	:	Human immunodeficiency virus
In-111	:	Indium-111
In-113m	:	Indium-113m
InhA	:	Enoyl-ACP reductase
KasA	:	β-keto acyl-ACP synthase
LE	:	Labelling efficiency
NaOCl	:	Sodium hypochlorite
PTB	:	Pulmonary tuberculosis
R or RIF	:	Rifampicin
RBC	:	Red blood cells
ROS	:	Reactive oxygen species
SNT	:	Supernatant
Sn^{2+}	:	Stannous ions
SOD	:	Superoxide dismutase
TB	:	Tuberculosis

TBH : Tygerberg hospital

Tc-99m : Technetium-99m

TcO_4^- : Technetium-99m pertechnetate

WHO : World Health Organization

Z or PZA: Pyrazinamide

5-ALA : Delta-amino levulinic acid

CHAPTER ONE
INTRODUCTION

Tuberculosis (TB) is a systemic infection caused by Mycobacterium tuberculosis. It is transmitted by coughed aerosol and usually presents with respiratory symptoms (Grandjean & Moore, 2008:454, Pai & O'Brien, 2008:560). While pulmonary tuberculosis is the most common presentation, tuberculosis can spread to virtually any tissue or organ of the body by way of haematogenous or lymphatic dissemination or contiguity (Jeong & Lee, 2008:834). Worldwide, approximately 8 million people develop active TB and 3 million people die of TB each year. South Africa is one of the 22 countries worst affected by tuberculosis (Dolin et al., 1994:213, Kirande, 2000:336), contributing at least 15% of the total tuberculosis caseload for Africa while accounting for only 7% of the continent's population.
Standard treatment of tuberculosis consists of isoniazid, rifampicin, pyrazinamide and ethambutol for two months followed by isoniazid and rifampicin alone for a further four months (Houston et al., 1991:252). The patient is considered cured at six months; relapse rate is 2 to 3%.

The ability of technetium-99m (Tc-99m) to become attached to red blood cells (RBC) that have been exposed to stannous ion has been known for over four decades (Sampson, 1996:652). Tc-99m labelled red blood cells are easily prepared and are widely used to diagnose sites of bleeding, such as haemoptysis (a common complication of pulmonary tuberculosis) or gastro-intestinal haemorrhaging, to calculate the left ventricular ejection fraction of the heart and to determine blood volume. RBC labelling with Tc-99m can be done using an *in vitro* technique, by *in vivo* methods, or by a combination of these two, sometimes called *in vitro/in vivo* labelling (Ryo et al., 1976:133-136). The labelling efficiency (LE), that is the extent to which the radionuclide is bound to the cells, is an important factor in the choice of labelling method. Using the *in vitro* technique, higher labelling efficiencies are achieved when compared to *in vivo* methods. The method of choice when labelling RBC with Tc-99m, *in vitro* versus *in vivo*, depends on the nuclear medicine examination requested. When a high labelling efficiency (higher than 90%) is imperative in order to obtain an accurate result, e.g. in the case of blood volume studies, the more laborious *in vitro* technique is used. When sufficiently accurate results can be obtained with slightly lower LE as is the case when calculating the left ventricular ejection fraction of the heart, *in vivo* labelling suffices.

Various drug therapies, including antibiotics, are known to either inflict direct or indirect damage to RBCs or their precursors or to impact influx or efflux of Tc-99m-pertechnetate into or out of RBCs, thereby decreasing LE to such an extent that poor and inaccurate diagnostic information is obtained. For instance, when using the *in vivo* method, drug-induced reduction of LE will result in higher plasma levels of unbound Tc-99m-pertechnetate which will be taken up or handled by the thyroid, kidneys and stomach. Uptake of unbound Tc-99m-pertechnetate in the stomach or kidneys may be misinterpreted as haemorrhaging in the gastro-intestinal tract (GIT) (false positive) and result in incorrect patient management.

In view of the high incidence of TB in South Africa, and particularly the Western Cape, antibiotics are administered daily to many patients undergoing nuclear medicine procedures that require red blood cell labelling. In this thesis, the effect of anti-tuberculosis medication on Tc-99m RBC labelling efficiencies is studied.

The **AIM** of the study therefore is to establish if the presence of anti-TB medication in venous blood has an effect on Tc-99m RBC labelling efficiencies when using an *in vitro* RBC kit. This will be done by comparing the labelling efficiency of Tc-99m to red blood cells in drug free tuberculosis patients, versus the labelling efficiency in the same patients when exposed to anti-tuberculosis drug loading over time.

CHAPTER TWO
LITERATURE REVIEW

In this chapter, the clinical presentation of TB as well as its incidence and prevalence worldwide and in South-Africa are reviewed. Routinely used anti-tuberculosis agents are reviewed and their potential toxic side-effects on red blood cells highlighted. Finally, the basic principles of RBC labelling as well as drugs affecting the labelling efficiency of radiolabelled red blood cells are addressed and discussed.

2.1 Tuberculosis

2.1.1 Tuberculosis: causative agent, clinical presentation, incidence and prevalence

Tuberculosis is caused by Mycobacterium tuberculosis. This micro-organism is most commonly transmitted from an open case of pulmonary disease to other persons by way of infected droplet nuclei. The chronic form of tuberculosis is predominantly pulmonary. Most commonly the bacteria destroy parts of the lung tissue, and the tissue is replaced by fibrous connective tissue. The affected areas of the lung do not recoil well during expiration and the air is retained due to the inelasticity of the connective tissue (Tortora & Grabowski, 1997:745-746). While pulmonary tuberculosis is the most common presentation, tuberculosis can spread to virtually any tissue or organ of the body by way of haematogenous or lymphatic dissemination or contiguity (Jeong & Lee, 2008:835). The most common sites of extra-pulmonary tuberculosis (EPTB) involvement are lymph nodes, followed by pleural effusion, and bone and joint involvement (Rubin, 2001:316).

Worldwide, approximately 8 million people develop active TB and 3 million people die of TB each year. Both the highest number of deaths and the highest mortality per capita are in the Africa Region. In 2005, the estimated per capita TB incidence in Africa was stable or falling. Unfortunately, the slow decline in incidence rates per capita proved offset by population growth. Consequently, the number of new cases arising each year is still increasing in Africa. While socio-economic conditions such as homelessness, and overcrowded houses with poor sanitation may to some extent contribute to the increasing number of cases of TB, human immunodeficiency virus (HIV) is by far the single most important factor contributing to the increase in incidence of TB since 1990. For instance, in 1995, about one third of the 17 million HIV-infected people worldwide, were also co-infected with M. tuberculosis (Harries & Maher, 1996:29-30). When compared to an individual who is not infected with HIV, an individual infected with HIV has a 10 times increased risk of developing TB.

South Africa ranks fourth on the list of 22 high-burden TB countries in the world (Kirande, 2000:336), contributing at least 15% of the total tuberculosis caseload for Africa whilst accounting for only 7% of the continent's population. In 2007, a staggering 461 000 new cases of TB were reported in South Africa, corresponding to an incidence of 948/100 000 inhabitants (WHO report, 2009:145). With regard to HIV, around 5.7 million South Africans were living with HIV at the end of 2007 (WHO Fact Sheet, 2008:4). Considering that approximately one-third of these patients simultaneously suffer from TB, approximately 1.9 million people in South Africa are likely to suffer from both TB and HIV.

2.1.2 Anti-tuberculosis Drugs: Mechanisms, Pharmacokinetics and Pharmacodynamics

Standard treatment of tuberculosis consists of isoniazid (H), rifampicin (R), pyrazinamide (Z) and ethambutol (E) for two months followed by isoniazid and rifampicin alone for a further four months. All four agents are bacteriostatic in nature, inhibiting specific metabolic pathways which will result in termination of mycobacterium tuberculosis growth followed by stasis (bacteriostatic) or death (bactericide) (Zhang, 2005:118). All four drugs should be taken as prescribed in order to limit the risk of developing drug-resistance, failure to comply with and/or complete the anti-TB treatment may enable microbes to tolerate certain amounts of an antibiotic that would normally inhibit them.

The basic pharmacokinetics and pharmacodynamics as well as side-effects of anti-TB drugs will be briefly addressed below.

Isoniazid (INH or H)

Isoniazid (isonicotinic acid hydrazide) is a highly effective anti-tuberculosis agent, that was first introduced in 1952 (Zhang, 2005:119, Cole, 1981:137). Its mechanism of action is the blockage of mycolic acid synthesis by inhibiting enoyl-ACP reductase (InhA) and/or β-keto acyl-ACP synthase (KasA). The route of administration of isoniazid is nearly always oral. After approximately 1 to 2 hours after an oral dose of 300 mg isoniazid, it is well absorbed from the gastrointestinal tract, achieving serum concentrations between 3 and 5 µg/ml. It is distributed into all body fluids and is only minimally bound to plasma proteins. Isoniazid crosses the placenta and achieves levels in milk comparable to those in maternal serum. It is metabolised by acetylation in the liver to acetyl isoniazid by the enzyme N-acetyltransferase and excreted in the urine as its metabolites, acetylisoniazid and isonicotinic acid. More specifically, approximately 75% – 95% of isoniazid is excreted in the urine over 24 hours as isoniazid and its metabolites (Friedman, 2001:306, Cole, 1981:137).

The most important adverse effect of isoniazid is hepatotoxicity. Additional side-effects include Pellagra, a skin disease resulting from vitamin B3 deficiency (Sweetman et al., 2007:257) and systemic lupus erythmatosis (Cole, 1981:140). Finally, in addition isoniazid may cause granulocytosis as well as haemolytic (auto-immune mediated) and sideroblastic anaemia. Sideroblastic anemia results from the direct inhibition of the amino-levulinic acid synthetase (ALA-S) by isoniazid. ALA-S condenses glycine and succinyl-CoA to form delta-amino levulinic acid (5-ALA) inside the mitochondria; the first step in heme-synthesis. Delta-amino levulinic acid subsequently moves to the cytosol where a number of additional enzymatic transformations produce coproporphyrinogin III. Coproporphyrinogin subsequently enters the mitochondrion where a final enzymatic conversion produces protophorphyrin IX. Finally, ferrochelase inserts iron into the protophorin IX ring to produce heme. As 5-ALA is not formed, body iron cannot be inserted into heme but becomes abnormally deposited in red blood cells which makes them unable to function properly.

Pyrazinamide (PZA or Z)

The bacteriostatic potential of pyrazinamide was recognised as far back as 1952. The mechanism of pyrazinamide is not known exactly, but it has a very narrow spectrum of activity. It is active against M. tuberculosis, but not against M. bovis (a closely related member of M. tuberculosis complex) or non-tuberculosis myobacteria. It is well absorbed by the gastrointestinal tract within 1 to 2 hours after oral administration. It is distributed into body tissues and fluids including cerebrospinal fluid. Pyrazinamide reaches plasma levels of 30 to 40 µg/ml, following a 1.5 g dose. Approximately 50% of pyrazinamide is bound to plasma proteins (Friedman, 2001:306). The metabolites of pyrazinamide, pyrazinoic and 5-hydroxypyrazinoic acids are filtered by the glomeruli and excreted in the urine. The unchanged drug, however, is reabsorbed from the renal tubules. Pyrazinoic acid suppresses the tubular secretion causing hyperuricaemia and occasionally gout (Cole, 1981:149). The most common adverse effects of pyrazinamide include jaundice, hepatitis, fever, anorexia, arthralgia and malaise (Friedman, 2001:307, Lambert & O'Grady, 1992:46-47).

Ethambutol Hydrochloride (E)

Ethambutol (discovered in 1961) is readily absorbed after oral administration. After a single dose of 25 mg per kg body weight, peak plasma concentrations of up to 5 μg per ml are obtained within 4 hours (Sweetman et al., 2007:245). Ethambutol appears to have a large volume of distribution which is due in part to active uptake of the drug by RBC (Friedman, 2001:312, Lambert & O'Grady, 1992:43); less than 10% of the drug is bound to plasma proteins. Approximately 1 hour after an intravenous dose two to three times as much ethambutol is present in the RBC when compared to plasma. RBCs thereby serve as a depot from which the medicine slowly enters the plasma.

An estimated 80% of the unchanged drug is excreted in the urine within the first 24 hours post administration (about 10-15% as an aldehyde and a dicarboxylic acid derivative), and approximately 20% of the drug is excreted in the faeces (Friedman, 2001:312, Lambert & O'Grady, 1992:43).

Ethambutol is generally well tolerated. However, gastro-intestinal disturbances have been reported (Sweetman et al., 2007:245) as well as peripheral neuropathy, skin eruptions (Cole, 1981:148), pruritis, headache, malaise and fever (Friedman, 2001:312). The most important side effect that results from treatment with ethambutol is retrobulbar neuritis (Cole, 1981:148, Lambert & O'Grady, 1992:43). In spite of the high levels of ethambutol inside RBCs, anaemia as side-effect is extremely rare.

Rifampicin (RIF or R)

Rifampicin (R) is considered to be bactericidal and is active against both intra-cellular and extra-cellular M. tuberculosis. It is an important agent in the treatment of tuberculosis, leprosy, and diseases caused by the non-tuberculous mycobacteria. Rifampicin inhibits the activity of DNA-dependent RNA polymerase (the enzyme responsible for catalysing the polymerisation of ribonucleotides into RNA molecules), a step which transfers genetic data from DNA to RNA. Rifampicin is well absorbed from the gastrointestinal tract, although the presence of food decreases absorption. Approximately 80% of the drug is bound to plasma proteins. Rifampicin concentrates in both tuberculous cavities as well as in cerebrospinal fluid in the setting of inflamed meninges (Friedman, 2001:308). The drug is widely distributed in all body tissues and due to its lipid solubility, it is able to penetrate cells and destroy intra-cellular micro-organisms. Reported side-effects include eosinophilia, leucopaenia, purpura and haemolysis (Sweetman et al., 2007:290-291). Haemolysis is related to the presence of rifampicin-dependent antibodies which bind complement to the patient's own red cells and is found in patients receiving high-dose intermittent rifampicin therapy. Other adverse effects associated with rifampicin are gastrointestinal disturbances as well as disturbances of hepatic function. Thrombocytopenia,

associated with complement fixing serum antibody, may also occur, as well as acute renal failure , sometimes associated with acute haemolysis (Lambert & O'Grady, 1992:265-266). Finally, rifampicin is a potent inducer of hepatic microsomal enzymes and its use in combination with drugs such as warfarin derivatives, oral contraceptives, corticosteroids, digoxin,verapamil and cyclosporine may result in decreased activity of these drugs (Friedman, 2001:309).

With regard to the aim of this thesis, it is noted that 2 drugs in particular have documented side-effects on RBCs, respectively isoniazid and rifampicin. Accordingly, these agents might theoretically affect binding of Tc-99m to red blood cells in a negative manner.

2.2 Red blood cell labelling

2.2.1 Red blood cell anatomy

Red blood cells are biconcave, nonnucleated discs that range from 7 to 8 μm in diameter, with a lifespan of approximately 110-120 days. RBC comprise the following: water (65%), haemoglobin (32%) and 3% other protein and lipid stroma (Srivastava & Chervu, 1984:68). These components of the RBC are enwrapped by a thin membrane that largely consists of proteins, as well as lipids. This membrane is partially permeable, therefore only specific components of the RBC are able to progress across the membrane and in turn only specific substances are allowed to penetrate the RBC via the membrane. Water and anions such as Cl^-, HCO_3^-, OH^- and $PO_4^{\equiv}$ are fully permeable, whereas haemoglobin, plasma proteins and cations such as K^+, Ca^{++}, Mg^{++} and H^+ are non-permeable (Brink, 1959:17). The most important component of the RBC, the haemoglobin, is an oxygen-carrying protein, that contains four haeme and globin molecules. Each RBC contains approximately 280 million haemoglobin molecules (Tortora & Grabowski, 1996:558-559). Haemoglobin and other intracellular components, as well as cell membrane proteins, participate to different degrees in binding various radiolabels to the RBC (Srivastava & Chervu, 1984:68).

2.2.2 Red blood cell labelling background

Radioactive labelling of red blood cells using Tc-99m was first reported in 1967 by Fischer et al. (1967:229). Various other radionuclides, such as indium-111 (In-111), indium-113m (In-113m), gallium-68 (Ga-68), chromium-51 (Cr-51) and technetium-99m were shown to be useful for labelling RBCs (Srivastava & Chervu, 1984:69, Sampson, 1990:216). Out of these radionuclides, the most frequently used are Cr-51 and Tc-99m. Due to the long half-life of Cr-51 (27.7 days), RBC can be labelled in order to determine the life span of red blood cell (RBC survival), splenic sequestration, as well as detecting small amounts of intermittent melaena due to gastro-intestinal (GIT) haemorrhaging. Gamma camera imaging is, however, not feasible when using Cr-51, due to the low yield of the gamma rays emitted (320 keV, 9% yield). However, measurements can be obtained using various detectors, such as wholebody counters, gamma probes and well counters. Technetium-99m (yielding gamma rays of 140 keV and a physical half-life of 6.02 hours), on the other hand, has ideal physical properties that makes it the radionuclide of choice for gamma camera imaging studies. The diagnostic applications using radiolabelled red blood cell imaging encountered nowadays in Nuclear Medicine are blood pool imaging (cardiac studies), detection of gastro-intestinal bleeding as well as pulmonary bleeding (haemoptysis), detection of haemangiomas and the detection of vascular malformations. Heat damaged radiolabelled red blood cells are also extremely useful in localising accessory spleen tissue. For determining blood volume and specifically red cell volume (red cell mass), technetium-99m is the isotope of choice, although for the latter indication no imaging is involved, but rather the detection of the radioactivity in blood samples by means of a well counter and then quantification of the data obtained (Srivastava & Chervu, 1984:69). Related to this project, only Tc-99m is of relevance. Accordingly, the labelling procedures of RBC with Tc-99m pertechnetate as well as drugs that might interfere with it will be now discussed in depth.

2.2.3 General labelling principle and the different labelling procedures

Red blood cell labelling with Tc-99m is based on the following principle:

- Stannous ions (Sn^{2+}) diffuse into the red blood cells and bind to the cellular components ("pre-tinning" of red blood cells).
- Tc-99m TcO_4^- (pertechnetate) freely diffuses back and forth across the cell membrane of red blood cells.
- In the presence of Sn^{2+}, pertechnetate is reduced and the technetium is then able to bind to the globulin (β-chain) of haemoglobin.

- Sn^{4+} and reduced forms of Tc-99m are normally not transported across the cell membrane.
- In the presence of extracellular Sn^{2+}, Tc-99m-pertechnetate will be reduced prior to entering the red blood cell and therefore be unable to cross the cell membrane. This inabillity to penetrate the red blood cell in turn will result in poor labelling of red blood cells (Srivastava & Chervu, 1984:76).

The principle of red blood cell labelling as described above, applies when labelling blood *in vitro*, *in vivo* as well as *invivitro*.

(i) In vitro red blood cell labelling

Labelling red blood cells *in vitro* with Tc-99m-pertechnetate, requires the use of a kit containing stannous ions, usually in lyophilized form. One to three millilitres of heparinised blood is added to the kit, and a 5 minute incubation period on an automatic rotator is allowed in order for the stannous ions (Sn^{2+}) to enter the RBC and to bind to the cellular components. This is followed the addition of sodium hypochlorite (NaOCl), which is an oxidizing agent (Kelbaek et al., 1986:1770). NaOCl is unable to cross the RBC membrane and will therefore be restricted to only oxidize the extra-cellular Sn^{2+} to Sn^{4+}. In order to remove the excess tin in the extra-cellular fluid, a citric acid/sodium citrate mixture (ACD-A) is added to sequester any residual extra-cellular stannous ion.

Typically, a red blood cell in vitro kit contains 5 μg stannous chloride dihydrate ($SnCl_2.2H_2O$), 3.7 mg sodium citrate, 5.5 mg dextrose and 2.0 mg sodium chloride.

A recommended dose of 370-3700 MBq Tc-99m is added to the kit and an incubation period of 20 minutes is allowed. The quantity of Tc-99m added to the red blood cells will depend on the clinical application, e.g. RBC labelled for blood volume studies will need only 60-80 MBq of Tc-99m, whereas blood labelled for GIT bleeding and haemoptysis studies need a larger amount of 1250-3000 MBq. The kit is centrifuged after labelling and plasma removed from the packed RBC. Any unbound Tc-99m will be removed with the plasma. The labelling efficiency is determined by measuring the activity in the supernatant as well as the activity in the RBC pellet. The following formula is used to calculate the percentage Tc-99m incorporated by the RBC (labelling efficiency):

$$\%LE = \frac{\text{activity of RBC}}{\text{activity of RBC + activity of SNT}} \times 100$$

The RBC pellet is resuspended in 0.9% saline solution and the preparation is now ready for injection. Labelling efficiencies of 95% or greater can be obtained with this method (Early & Sodee, 1995:109).

(ii) In vivo red blood cell labelling

This method of radioactively labelling red blood cells entails reconstituting a vial of stannous pyrophosphate with sterile 0.9% saline solution or sterile water for injection. An amount of 10-20 μg of stannous ion per kilogram body weight is thought to be adequate (Srivastava & Chervu, 1984:75). The solution is intravenously administered to the patient and a 30 minute waiting period is given in order to allow the "pre-tinning" of the red blood cells. Tc-99m pertechnetate is then injected intravenously after the incubation period, and RBC labelling occurs almost immediately in the circulating blood. Removing excess stannous ions, as well as determining the labelling efficiency of red blood cells prior to administration, is not possible when utilizing this method (Sampson, 1990:214). Early and Sodee state that only about 75% of radioactivity is taken up by the RBCs when labelling *in vivo*, whereas the in vitro method yields greater labelling efficiencies (1995:109). The exact labelling efficiency cannot be determined with this method and therefore it is not useful in quantitative studies. In a study performed on rats by Hamilton and Alderson (1977:1011), it was found that approximately 90% of the injected dose remained in the blood pool 30 minutes to 2 hours post in vivo RBC labelling.

(iii) In vivitro red blood cell labelling

This quantitative method for red blood cell labelling is a combination of the *in vivo* and *in vitro* methods. The red blood cells are "pre-tinned" in vivo, but labelled in vitro, in order to remove unbound Tc-99m prior to injection. This method also allows the measurement of the radioactivity and therefore the calculation of the LE (Sampson, 1990:215). This variation of the *in vivo/in vitro* method as described by Dacie and Lewis (in Sampson, 1990:215-216) entails the reconstitution of a stannous agent with 0.9 % sterile saline solution, followed by an intravenous injection of 0.03 ml/kg of the stannous ion. After a waiting period of 15-30 minutes a 10 ml heparinised blood sample is collected of the *in vivo* tinned blood. Sterile saline (0.9 %) is added to the

blood and then centrifuged for 5 minutes at 500 gravitational force (g). The plasma is then removed and the washing step is repeated, to ensure that any extra-cellular stannous ions have been removed from the cells before the addition of the pertechnetate. The required amount of pertechnetate is added to the washed RBC and is mixed for 5 minutes. This is followed by the addition of 0.9 % sterile saline and centrifugation for 5 minutes at 1200-1500 g. The supernatant is removed and the labelling efficiency is determined as described in 2.2.3 (i).

Modified *in vivo* RBC labelling methods can produce labelling efficiencies suffiecient to obtain accurate results (Srivastava & Chervu, 1984:75, Sampson, 1990:215). According to Early and Sodee (1995:110), these labelling methods can produce labelling efficiencies as high as 95%. Srivastava and Chervu describe this method to be an *in vivo* "tinning" procedure followed by presentation of Tc99m-pertechnetate activity to a smaller number of RBC *in vitro* and re-injecting the labelled RBC in plasma into the patient.

The method of choice for labelling RBC will largely depend on the indication for the referral of the patient. *In vivo* RBC is a convenient method, that eliminates the direct handling of blood and it is less time-consuming than the *in vitro* method. However the *in vivo* method generally produces poorer labelling efficiencies and therefore limits the usefulness of this method for a number of applications. When dealing with quantifiable application such as blood volume studies, it is imperative to ensure a high labelling efficiency. In the detection of haemoptysis, poor labelling efficiencies will result in uptake of the unbound radioactivity in the stomach, which in turn could result in false positive studies. Therefore, for the above mentioned studies, the in vitro method would take preference over all alternative methods of RBC labelling with Tc-99m.

2.2.4 Drugs affecting red cell labelling efficiency

Drugs can affect red cell formation and biochemistry and various reviews have shown that a large number of drugs can cause diseases of red cells, such as hemolytic anaemia, oxidative haemolysis, megaloblastic anaemia, mitochondrial damage and membrane disruption (Morse, 1989:13-18, Ammuse & Yunis, 1989:71-82).

According to Sampson (1998:529) it has become apparent in recent years that the patient's drug therapy can affect the radiolabelling of both white and red cells to such an extent that only poor diagnostic information is obtained. In some patients, RBC antibody formation may be the primary factor causing poor radiolabelling. It is believed that drug therapy may be partially responsible for this RBC antibody formation (Hladik et al., 1982:202). Leitl et al. (1980:P44) found RBC labelling

efficiencies to be poor (less than 50%, compared to a mean value of greater than 90%) in 8 of 40 consecutive patients. Each of these 8 patients had disease or were receiving medications associated with RBC antibody formation.

Sampson (1993:280-294) stated that significantly decreased labelling efficiencies were found in patients using prazosin and digoxin. Other drugs such as propranolol, verapamil and furosemide also had meaningful effect. In another article Sampson reported that patients who were taking nifedipine in combination with other drugs, yielded sub-optimal labelling efficiencies and it is suspected that drug interaction could be a possible cause (Sampson, 1992:229).

Sampson (1996:653) gives a summary of documented causes of poor labelling efficiency of Tc-99m RBC's, several of which are drug-related:

- Heparin anti-coagulant rather than acid-citrate dextrose solution A.
- Oxidation of Tc-99m with exposure to air;
- Inadequate administration of stannous ion;
- Prior administration of intravenous contrast media;
- Patients with sickle cell anaemia;
- Incubation with anti-RhD serum;
- Anti-hypertensive therapy;
- Formation of RBC antibodies;
- Interference of drugs such as nifedipine, verapamil.

Antibiotics such as gentamycin and anti-inflammatory agents may also affect the labelling efficiency (Gleue et al., 1995:22).

Oliveira and colleagues researched a wide range of other factors that could potentially adversely affect RBC labelling. Most of the experiments done were *in vitro* incubation of various extracts of plants in specific concentrations. The yield of Tc-99m RBC labelling decreases in the presence of extracts with oxidant properties. They found that natural products such as Thuya occidentalis (Oliveira et al., 1997:489-494) tobacco extract (Vidal et al, 1998:1-6), as well as Maytenus ilicifolia extract (Oliveira et al., 2000:179-184) are able to interfere with Tc-99m RBC labelling, with histological alterations of the red blood cells as a possible cause.

Kelbaek et al. (1986:1772) found that the addition of 2 ml or more NaOCl to the RBC during *in vitro* labelling considerably decreased labelling efficiencies, due to haemolysis of the RBC.

Tatum et al. (1983:585-587) reported cases of suboptimal RBC labelling where patients had received intravenous administration of iodinated contrast media in the previous 24 hours.
Hambye et al. (1995:64-66) found poor tagging of RBCs and subsequently suboptimal image quality in patients treated with anthracycline chemotherapy for breast carcinoma. The image quality did not improve until three months after cessation of therapy. This may be the result of circulating RBCs that have been exposed to toxicity, and that are still present in the patient's system after a few weeks post therapy.

Due to the extremely high prevalence of tuberculosis in South Africa of 692/100 000 population (WHO report, 2009:145), many patients undergoing nuclear medicine investigations (including those that require red blood cell labelling) are on anti-tuberculosis medication. Given the fact that literature suggests possible adverse effects of antibiotics on RBC labelling, and no studies were found that specifically dealt with anti-tuberculosis medication, it is necessary to determine whether anti-tuberculosis medication might also have this effect.
The anti-tuberculosis drug regimen is administered over a long period, where the RBCs are exposed to the cocktail of prescribed drugs. It is therefore necessary to determine over a prolonged period, what the effect of the drugs would be on RBC labelling efficiencies.

The effect of anti-TB medication on Tc-99m RBC labelling will be addressed in this study.

CHAPTER THREE
METHODS

Ethical approval for this research project was granted from the academic institutions of Cape Peninsula University of Technology as well as Stellenbosch University .

3.1 Sample collection

A convenience sampling approach was used for this study. All new pulmonary tuberculosis (PTB) patients, attending the clinics of Ravensmead and Uitsig in the Tygerberg metropole (Western Cape, RSA), were eligible.

Informed consent was obtained from each patient on enrolment into the study, by means of an information sheet explained and given to the patient. The information sheet was available in the 3 languages most applicable in the areas identified: English, Afrikaans and Xhosa (see Addendum D).

Inclusion criteria were:

- Consenting females and males aged between 18-55 years
- Bacteriological evidence of PTB (stained smears of sputum positive for M tuberculosis)
- Free from HIV
- No history of previous anti-TB treatment
- Pre-commencement of anti-TB treatment
- Patient compliance with treatment regimen

Exclusion criteria were:

- On request of the patient
- Previous exposure to anti-TB drugs
- Patients on drugs known to interact with anti-TB drugs
- Patients with psychiatric illness
- Dysfunction not due to HIV infection
- Patients with chronic diseases
- Critically ill patients

3.2 Tuberculosis control programme drug regimen

The anti-TB drug regimen implemented in South Africa runs a course of 6 months (Western Cape Academic Hospitals Antibiotic Recommendations, 2003:19).

The first *two months* of treatment comprises a cocktail of 4 drugs:

- Rifampicin (R)
- Isonizid (H)
- Pyrazinamide (Z)
- Ethambutol (E)

The remaining *4 months* of treatment, a 2 drug combination is used:

- Rifampicin (R)
- Isoniazid (H)

The dosages given to patients are summarised in table 3.1.

Table 3.1: Anti-Tuberculosis Drug regimen

Pre-treatment Body weight	**Two months initial phase when given five times a week**	**Four months continuation phase when given five times a week**	
	RHZE (150, 75, 400, 275 mg)	**RH (150,75 mg)**	**RH (300,150 mg)**
30 – 37 kg	2 tablets	2 tablets	
38 – 54 kg	3 tablets	3 tablets	
55 – 70 kg	4 tablets		2 tablets
≥ 71 kg	5 tablets		3 tablets

3.3 Red blood cell labelling

Venous blood samples were collected from the enrolled patients using a 21 gauge (G) vacutainer Safety-Lok^{TM} blood collection set and a heparinised vacuum test tube.

These blood samples were collected at three different times during treatment, after the patient had been diagnosed with PTB.

1. **t0/baseline:** before commencement of anti-TB treatment
2. **1 month** after commencement of treatment: mid-point of intensive phase
3. **4 months** after commencement of treatment: mid-point of continuation phase

The *in vitro* red blood cell labelling procedure was utilised based on the Brookhaven method.

All samples were labelled in duplicate aliquots of 1 ml.

Method of Tc-99m RBC labelling

The Brookhaven method as described by Le Roux (1991:52-53) was used:

Duplicate 1 ml aliquots of the blood sample were added to an in-house prepared *in vitro* red blood cell kit. (Each kit contains 5 μg $SnCl_2.2H_2O$, 3.7 mg sodium citrate, 5.5 mg dextrose en 2.0 mg sodium chloride).

The RBC kit with blood was incubated for 5 minutes on an automatic rotator at room temperature.

After incubation, 0.6 ml of a 0.1% sodium hypochlorite (NaOCl) solution was added to the mixture, followed by 1 ml acid citrate dextrose solution-A (ACD-A).

A dose of 900-3000 MBq Tc-99m pertechnetate (fresh eluate) was then added to the kit.

The kit was incubated for 15 minutes. During this period the contents of the kit were mixed carefully every 5 minutes.

The kit was centrifuged at 2000 revolutions per minute, for 5 minutes.

The supernatant (SNT) was then removed from the red blood cells and placed in a separate container.

Measurement

The radioactivity of both the packed red blood cells (RBC) and the supernatant (SNT) was measured in a dose calibrator.

The labelling efficiency (LE) was calculated as follows:

$$\%LE = \frac{\text{activity of RBC}}{\text{activity of RBC + activity of SNT}} \text{ X } 100$$

The above described in vitro red blood cell labelling procedure was performed on all samples collected from the enrolled patients in the various stages of treatment.

Additional labellings were done on a control group of blood samples of normal drug-free volunteers. The labelling process was identical to that used for the enrolled patients.

3.4 Studies on drug-free blood from healthy volunteers

As the data collection and processing of patient samples progressed, questions arose regarding the normal variations to be expected in labelling efficiency. Furthermore, in some cases blood samples could only be labelled 30 or more minutes after blood collection. In order to strengthen the data analysis, samples were obtained from normal volunteers to study the abovementioned issues.

Normal variation in labelling efficiencies in consecutive samples when labelling RBC with Tc-99m

A single drug-free blood sample (15 ml) was taken from 3 healthy volunteers. The blood sample was divided into 9 sub-samples (of 1 ml each), to radioactively label 9 *in vitro* red blood cell kits.

The effect of time between sampling and radiolabelling

Red cell labelling of blood samples from volunteers was performed on 3 different times after the blood sample was taken, i.e. 20 min, 70 min and 150 min after the blood samples were drawn.

3.5 Data Processing and statistical analysis

Labelling efficiency with and without TB treatment

Average labelling efficiency and standard deviation for all patients were calculated for each treatment phase. Scatterplots were created and regression lines determined for each treatment phase. A repeated measures analysis of variance (RM ANOVA) was done on the % LE over months, at months 0 (baseline), month 1 and month 4. Since the residuals were not normally distributed, a non-parametric test was also done, i.e. the Friedman test.

The influence of factors other than the TB medication on labelling efficiencies

To ensure that any effects observed on study samples were not due to confounding factors, additional analyses of the data were performed.

Scatter plots were obtained and regression lines were fitted for the following factors at each treatment phase:

- time delay between sampling and radiolabelling of blood (time to labelling)
- age of the RBC kit (RBC age).
- Age of the NaOCl solution used during labelling (NaOCl age)

Then on all subsets, multiple regression analysis of % LE on the independent variables (time to labelling, NaOCl age and RBC kit age) was done with selection criteria: Adjusted R^2, Mallows' < p and step-wise regression procedure as well as univariate tests of significance was done.

Studies on normal volunteers

a) repeated labelling in different volunteers

RM ANOVA was done of LE in different volunteers

b)The effect of time between sampling and radiolabelling

A repeated measures analysis of variance (RM ANOVA) was done on the % LE over time. The experiment was done three times on the same subjects at times 20, 70 and 150 minutes.

CHAPTER FOUR
RESULTS

In this chapter, the results of the radiolabelling of patient samples with and without TB medication are shown. In addition, results of supportive studies in normal volunteers are shown. Statistical analysis of data was extended beyond the effect of TB medication only, in order to exclude possible effects of confounding factors.

The study required adult patients with newly diagnosed pulmonary tuberculosis (PTB).
A total of 45 patients were enrolled in the study and baseline studies (t0) were performed on their blood samples. Only 39 of the enrolled patients were able to be followed up 1 month into their anti-TB treatment and 33 patients managed to complete all three follow-up blood samples (up to 4 months into treatment) for RBC labelling. All blood samples (t0, 1 month and 4 months) were labelled in duplicate and their percentage labelling efficiency documented.

4.1 Patient studies

Full details of all patient blood samples, including time delay between obtaining the sample and radiolabelling, age of the RBC kit and NaOCl solution respectively, and LE are provided in Addendum A.

Labelling efficiencies for all patient samples are summarised in a table provided in Addendum B and shown in the graphs below (Figures 4.1A to 4.1E). Patient numbers are in chronological order and the grouping per 10 patients is done only for easier viewing of the graphs. From these graphs it can be seen that a wide variation in LE (ranging between 80 – 99%) was obtained, even within samples of a single patient. The biggest variation in LE of various samples labelled in a single patient at one time point, was 12.3% (patient 44). Out of 236 labellings done, LE's were above 90% in 219 of the cases and 17 were between 80% and 90%.

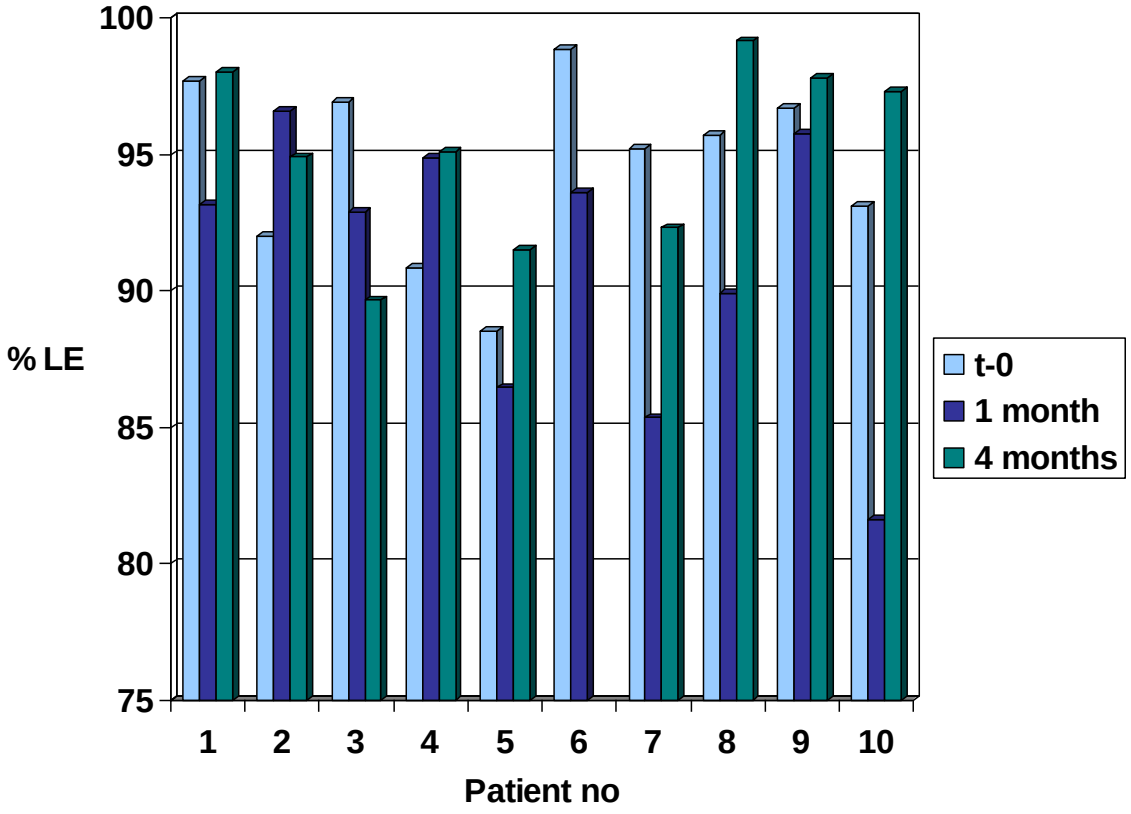

Figure 4.1 A: Labelling efficiencies for patients 1 to 10

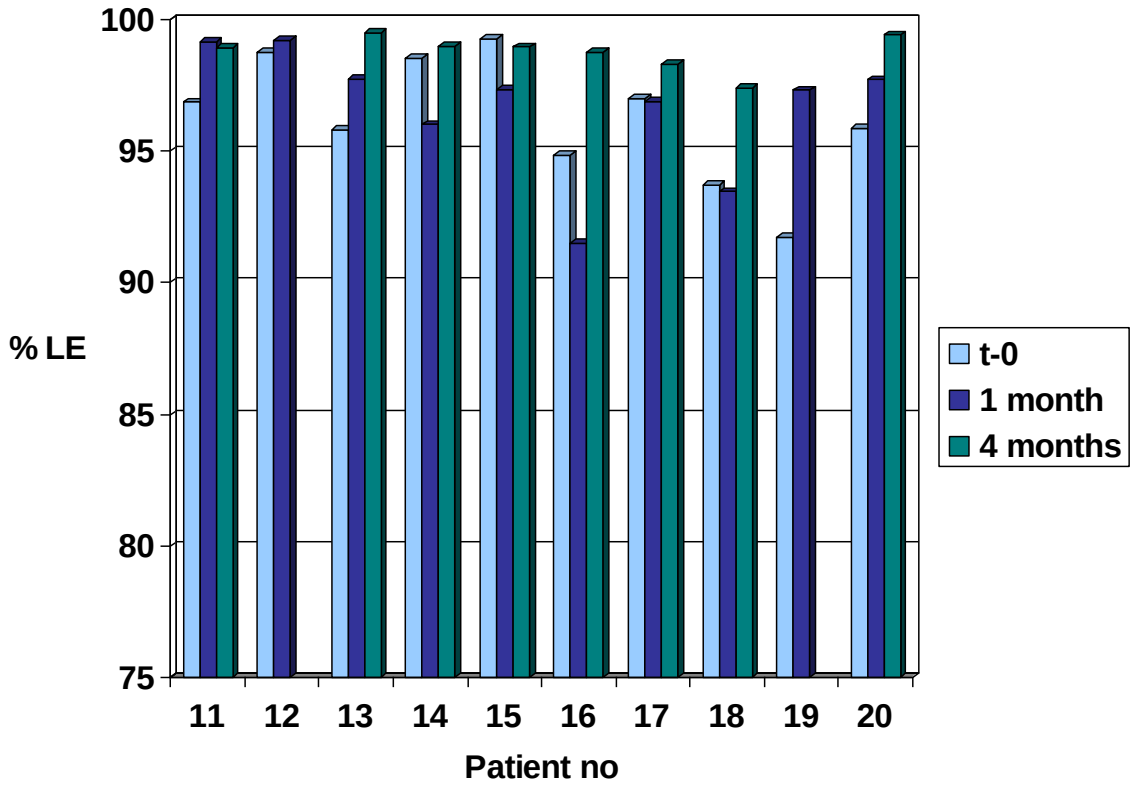

Figure 4.1 B: Labelling efficiencies for patients 11 to 20

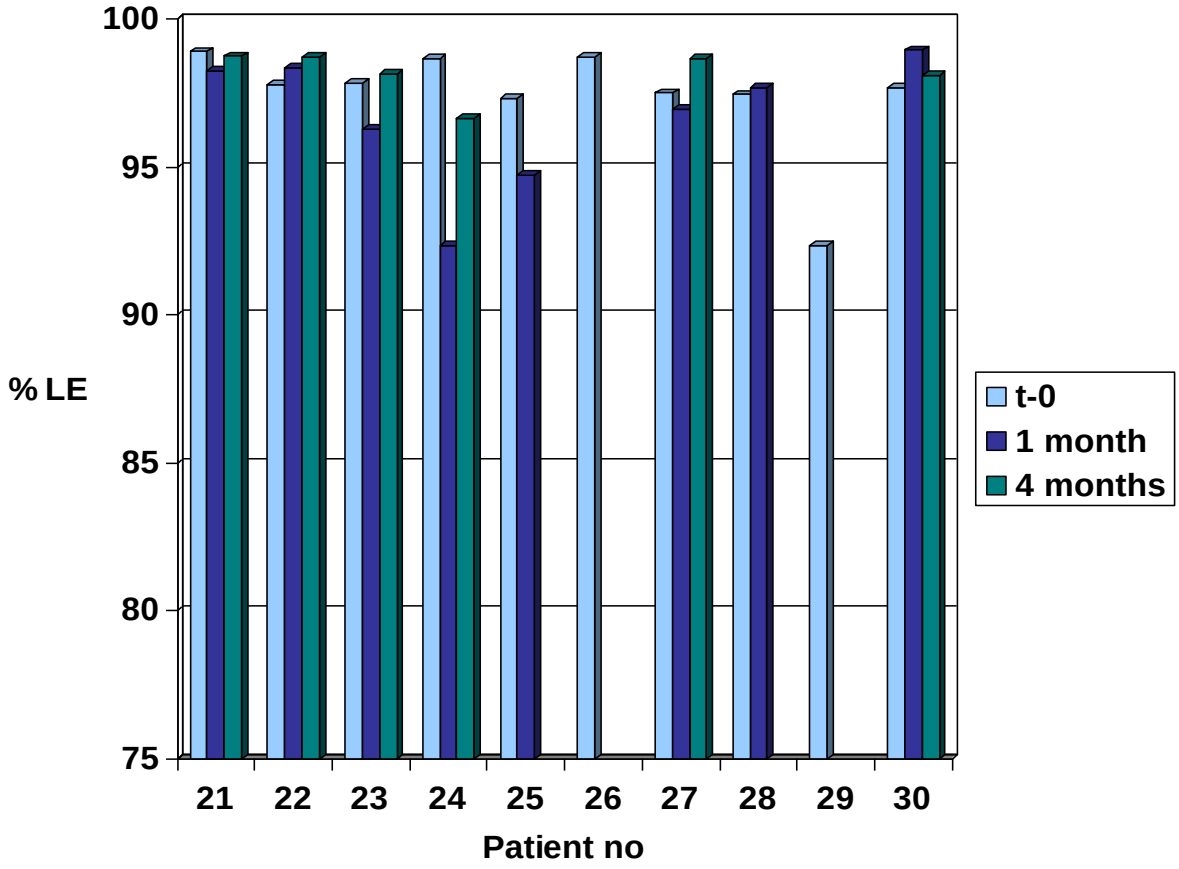

Figure 4.1C: Labelling efficiencies for patients 21 to 30

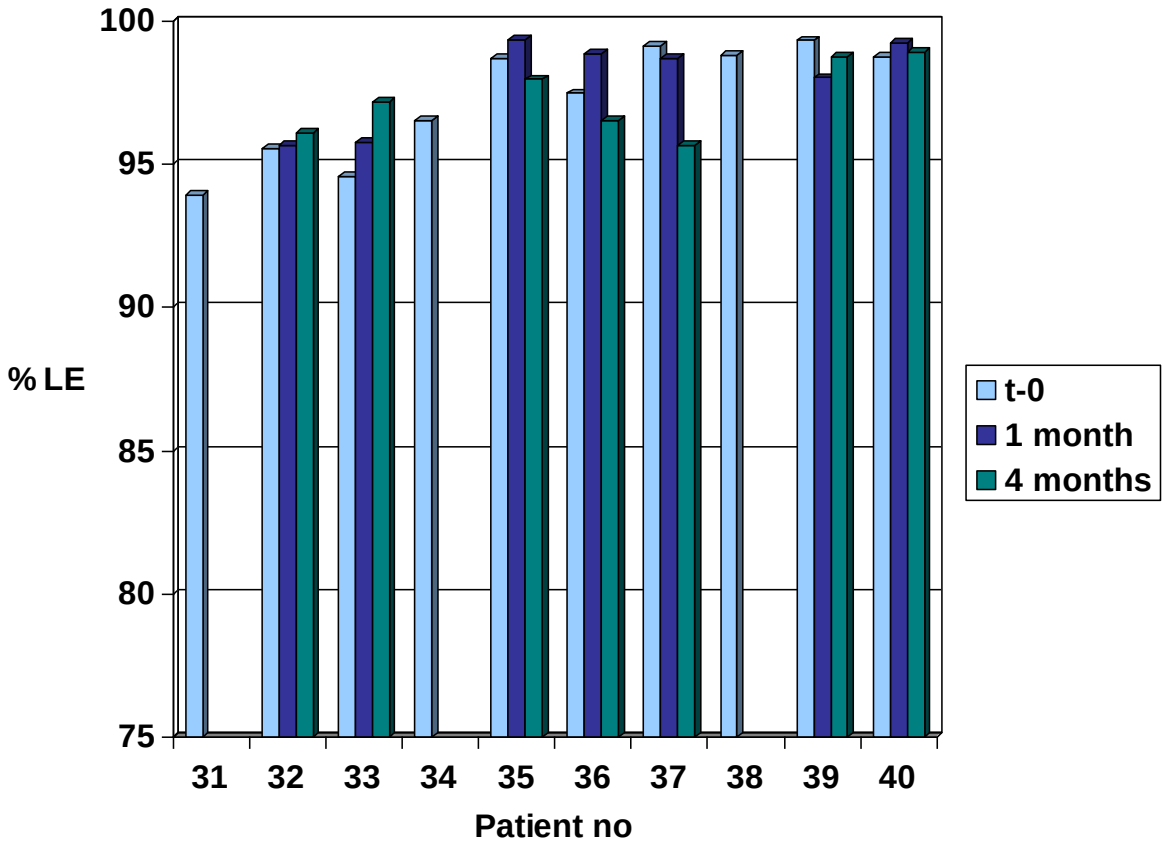

Figure 4.1 D: Labelling efficiencies for patients 31 to 40.

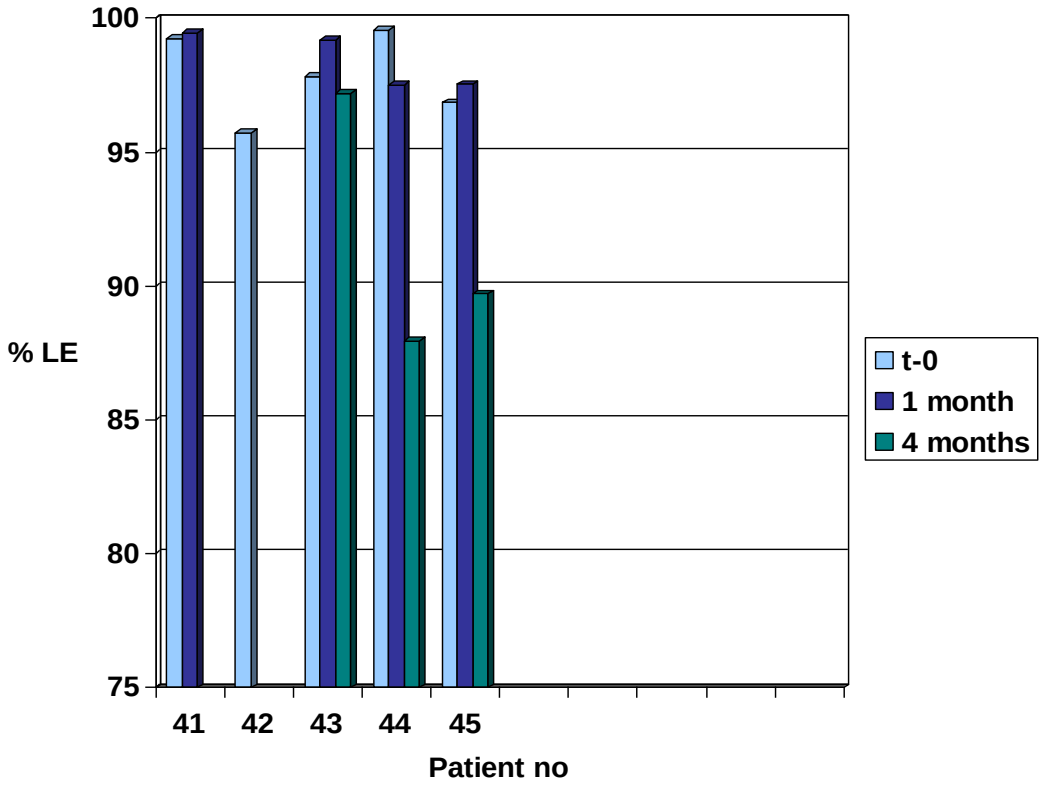

Figure 4.1 E: Labelling efficiencies for patients 41 to 45.

Statistical comparison of the three time phases was only done in the subgroup of 33 patients who had data for all three time points.

The average labelling efficiencies and mean standard deviation for LE obtained after duplicate samples were done in the various stages of their treatment regimen:

- **t0** : baseline study (before commencement of treatment) yielded an average LE of 96.5% with a standard error of 0.45%.
- **1 M**: 1 month after commencement of treatment (mid-point of intensive phase) yielded an average LE of 95.2% ± 0.75%.
- **4 M**: 4 months after commencement of treatment (mid-point of continuation phase) the average LE was 96.7% ± 0.52%.

These values are shown in figure 4.2

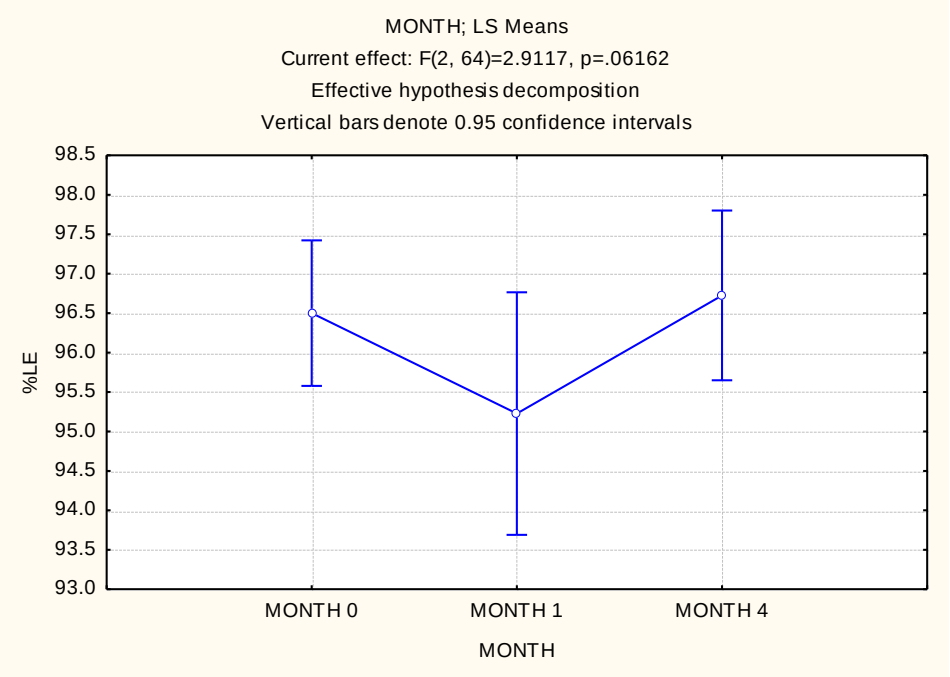

Figure 4.2: Means and 95% confidence intervals of LE at different stages of treatment

The Normal probability plots for the three different treatment phases showed that the residuals were not normally distributed (Figures 4.3 A to 4.3 C).

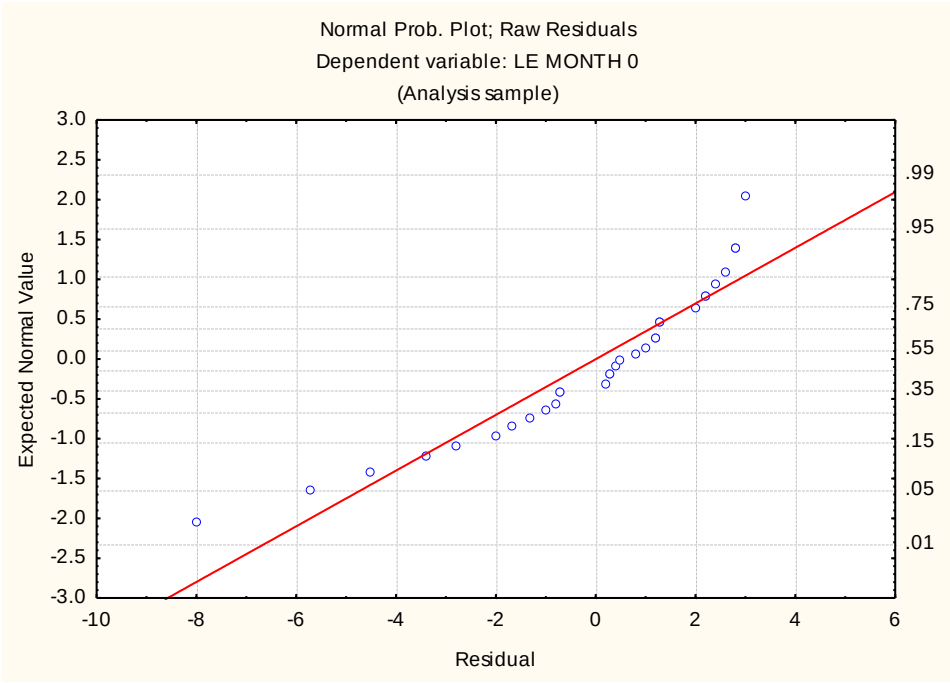

Figure 4.3 A: Normal probability plot Month 0

Figure 4.3 B: Normal probability plot Month 1

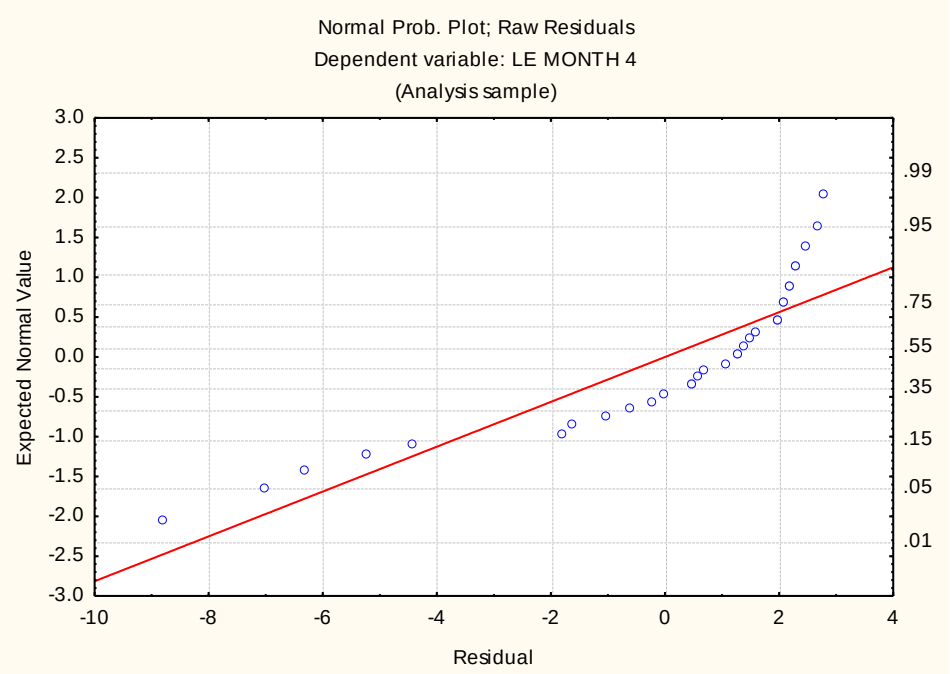

Figure 4.3 C: Normal probability plot Month 4

Labelling efficiency with and without TB treatment

A repeated measures analysis of variance (RM ANOVA) was done on the % LE at the different treatment phases, at month 0 (baseline), month 1 and month 4. The % LE was the lowest at month 1, but the % LE over the 3 treatment phases did not differ significantly ($F_{2.64}$ = 2.9117 with p-value p = 0.06162). Since the residuals were not normally distributed (see Normal Probability Plots at each month, figures 4.3 A to 4.3 C), a non-parametric test was done, i.e. the Friedman test. This test confirmed the non significance, with χ^2 = 5.328 and the p-value p = 0.06966.

4.2 Evaluation of variations in labelling efficiencies in samples from healthy volunteers

All details of labelling efficiencies obtained for samples from healthy volunteers are provided in Addendum C.

The red cell labelling of samples from healthy volunteers at 20 min, 70 min, and 150 min after the initial blood sample was drawn are shown in figure 4.1 and table 4.1, where V1 refers to volunteer 1, V2 is the second volunteer and V3 is volunteer 3.

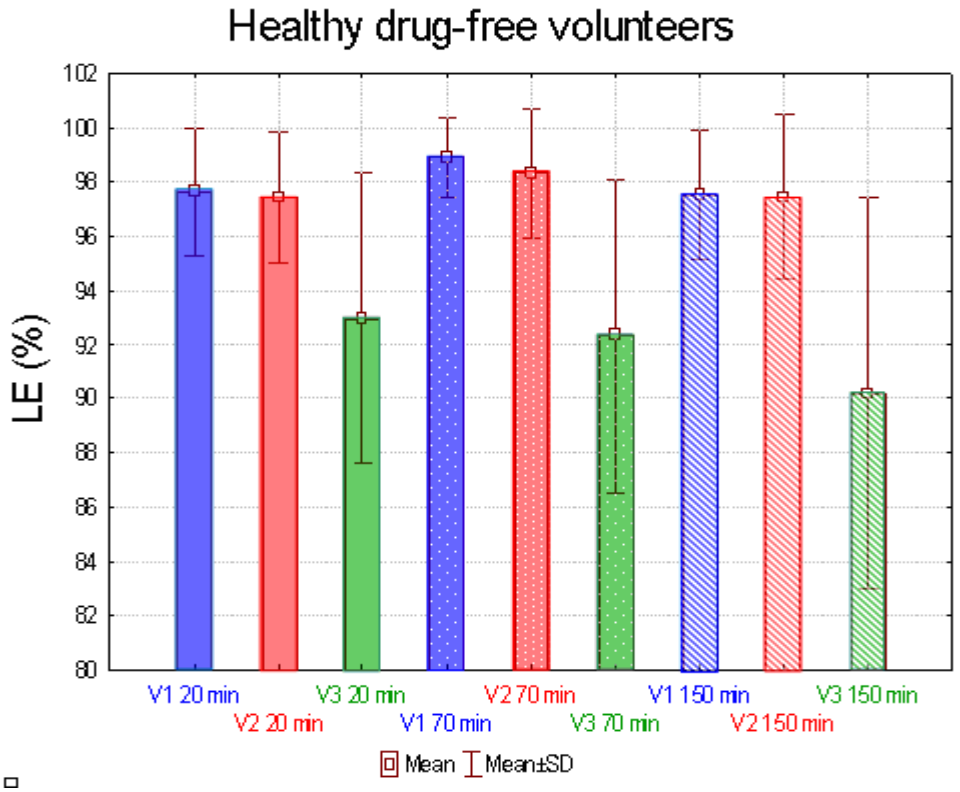

Figure 4.4: Normal variation in drug-free volunteers

Normal variation in labelling efficiencies in consecutive samples when labelling RBC with Tc-99m

Repeated labelling in the same volunteer showed standard deviations ranging from 0.4% to 2.4%.

From the graph (figure 4.4) it is clear that the mean LE from volunteer 3 (average LE's between 86.6% and 90.3%) differs significantly over time from those of volunteers 1 and 2 (average LE's all more than 96%) with p=0.0029.

The effect of time between sampling and radiolabelling

Repeated measures analysis of variance showed that the mean responses did not differ significantly over the different times (p=0.192).

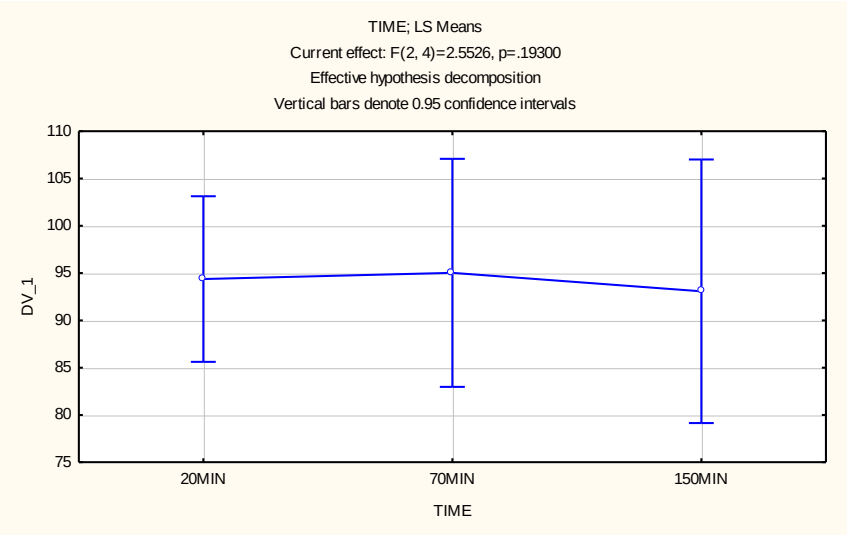

Figure 4.5: Average LE for normal volunteers at different times after blood collection

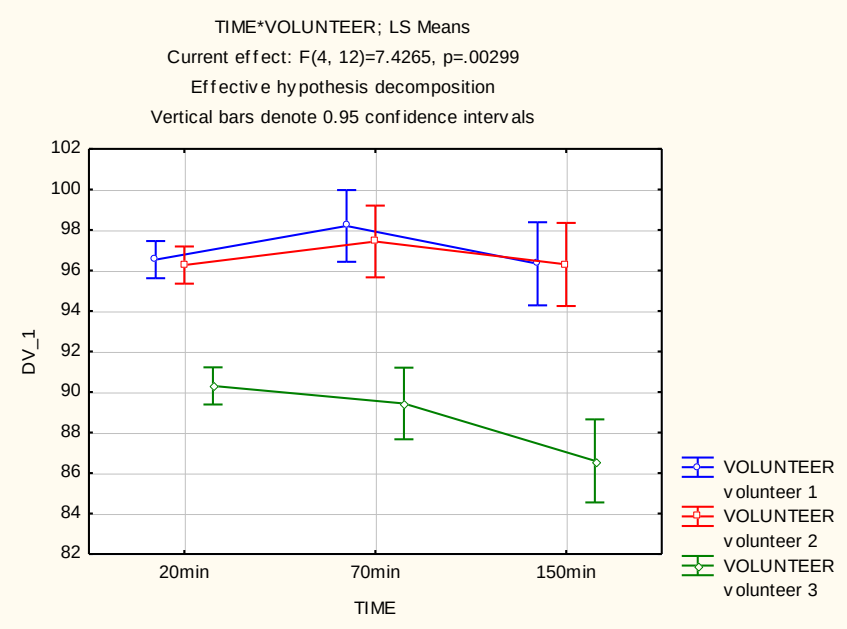

Figure 4.6: Normal volunteer analysis over time showing values for the different individuals

4.3 Evaluation of factors other than TB treatment on labelling efficiency

The scatterplots and regression lines for the different variables at each treatment phase are given below:

Time delay between sampling and radiolabelling of blood

The time delay between obtaining the blood sample and radiolabelling did not give significant regression at any treatment phase.

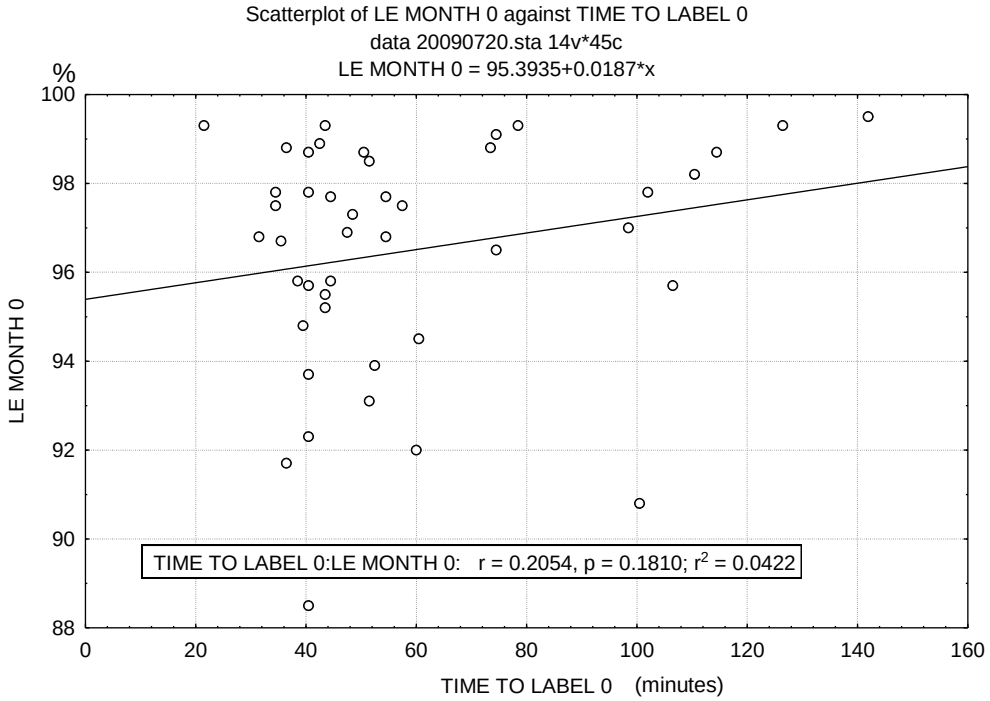

Figure 4.7 A: Scatterplot for Month 0 to show the effect of time delay between blood sampling and radiolabelling on LE

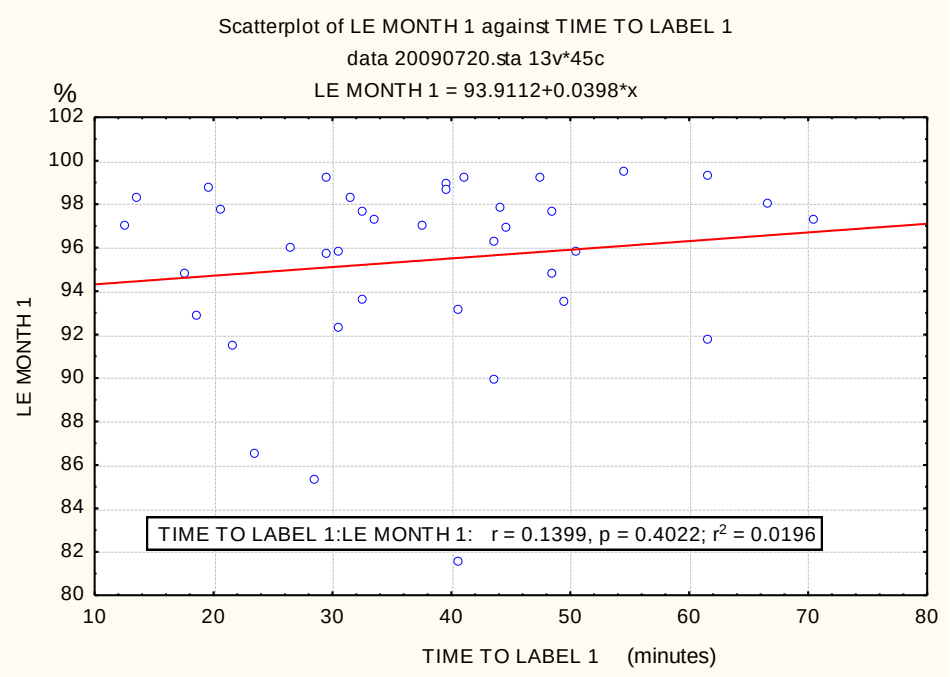

Figure 4.7 B: Scatterplot for Month 1 to show the effect of time delay between blood sampling and radiolabelling on LE

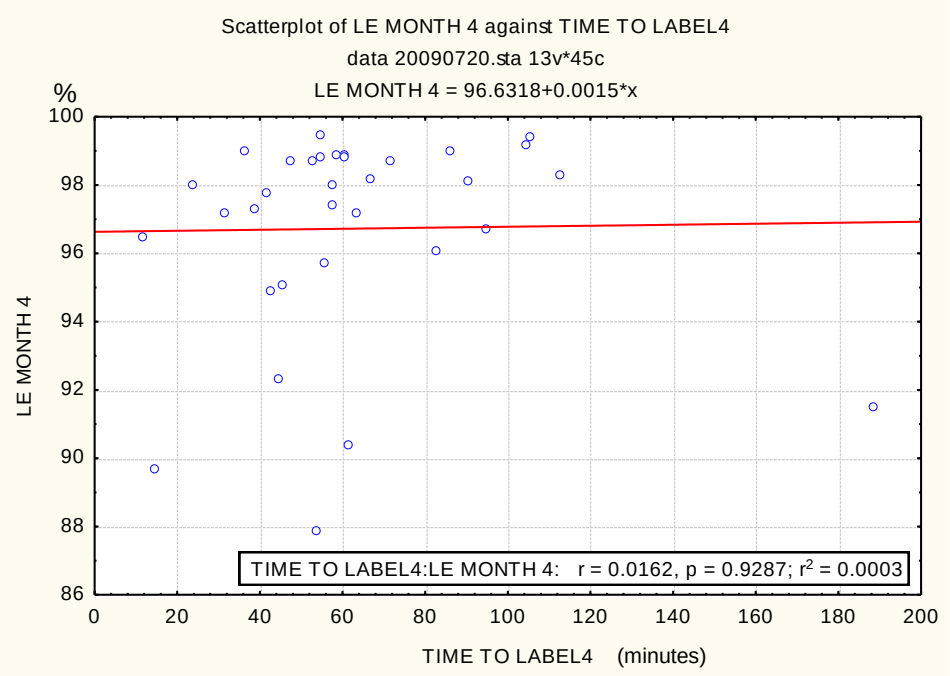

Figure 4.7 C: Scatterplot for Month 4: to show the effect of time delay between blood sampling and radiolabelling on LE

Age of the RBC kit:

Significant regression of the labelling efficiency was observed in t0 for the RBC age (univariate test of significance for LE, p = 0,0039) and for month 4 (p <0.0001).

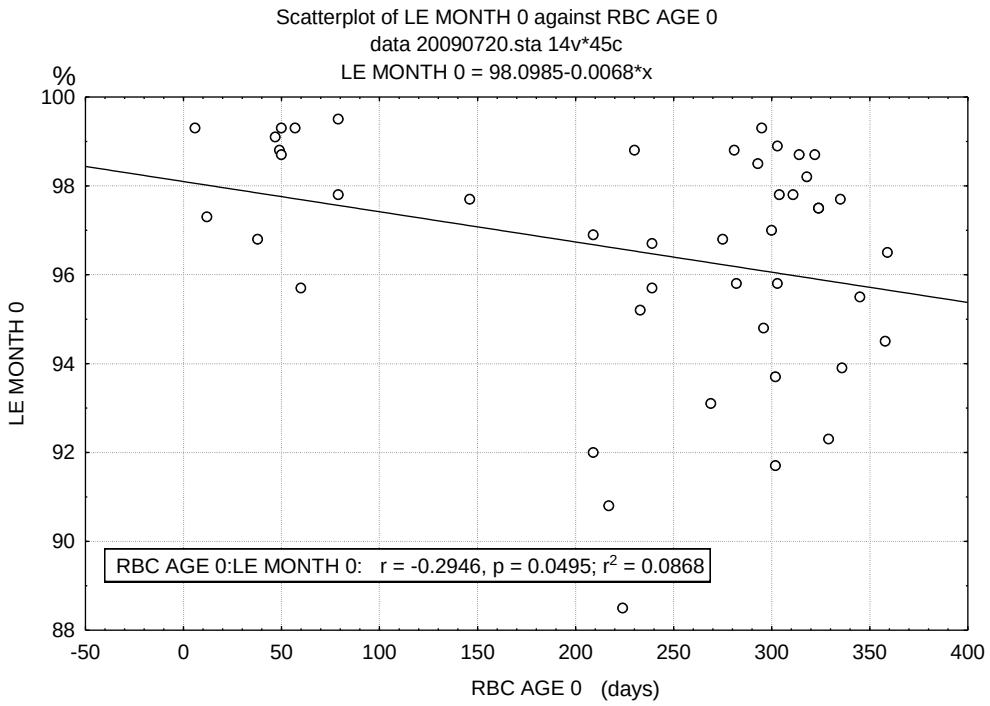

Figure 4.8 A: Scatterplot for Month 0 to show the effect of the age of the RBC kit on LE

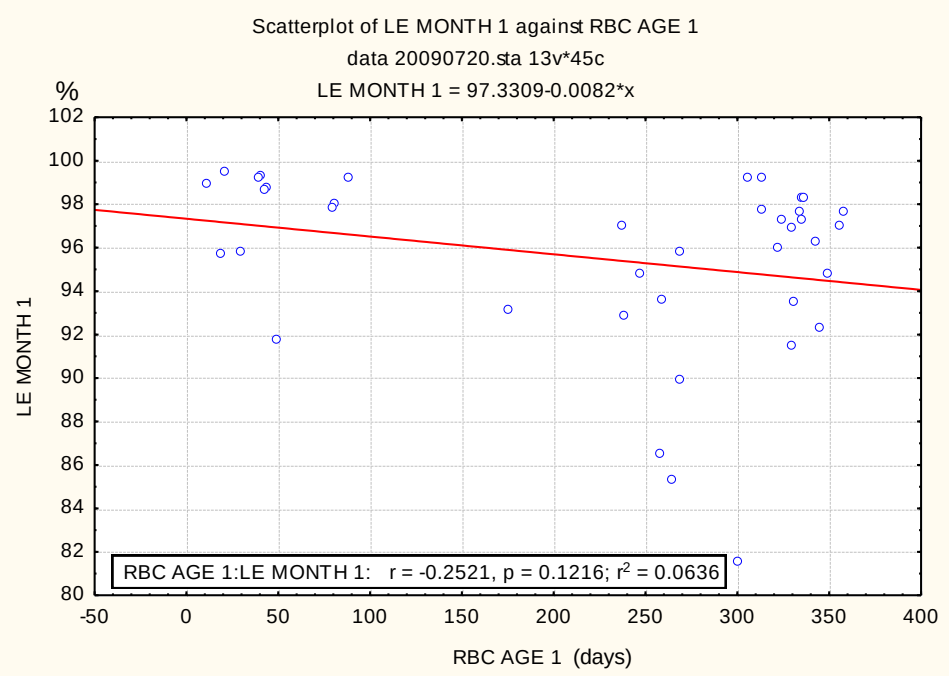

Figure 4.8 B: Scatterplot for Month 1 to show the effect of the age of the RBC kit on LE

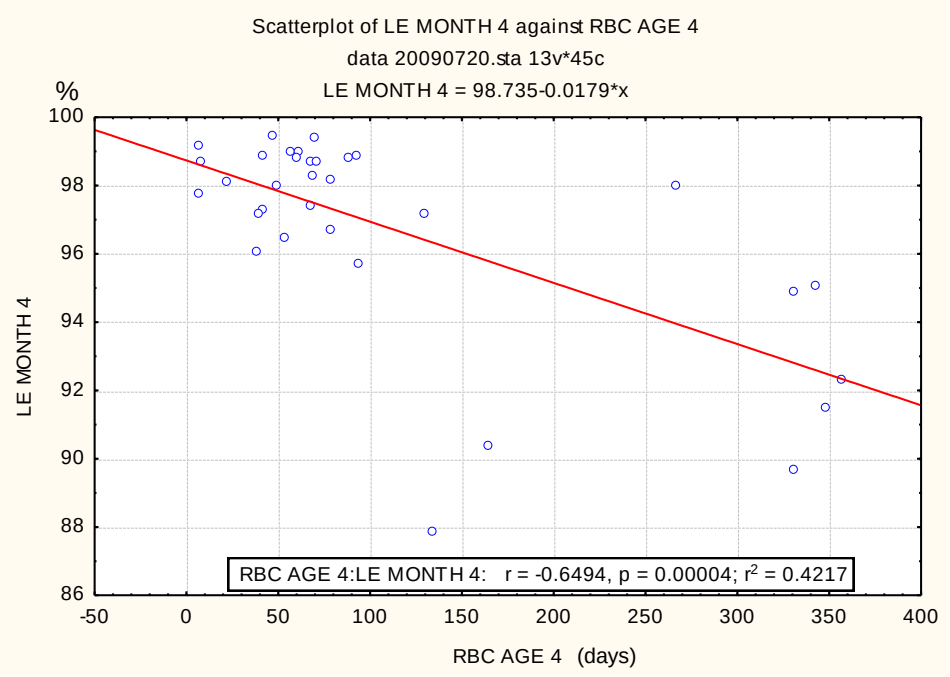

Figure 4.8 C: Scatterplot for Month 4 to show the effect of the age of the RBC kit on LE

Age of the NaOCl solution used during labelling

Significant regression of the labelling efficiency was only observed in t0 ($p < 0.0003$).

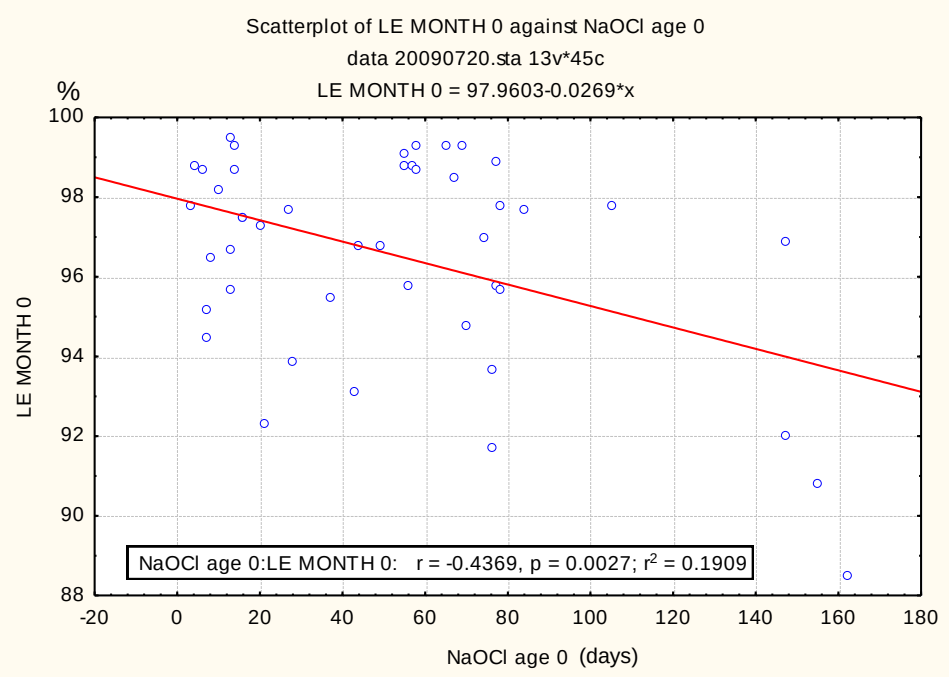

Figure 4.9 A: Scatterplot for Month 0 to show the effect of the age of the NaOCl solution on LE

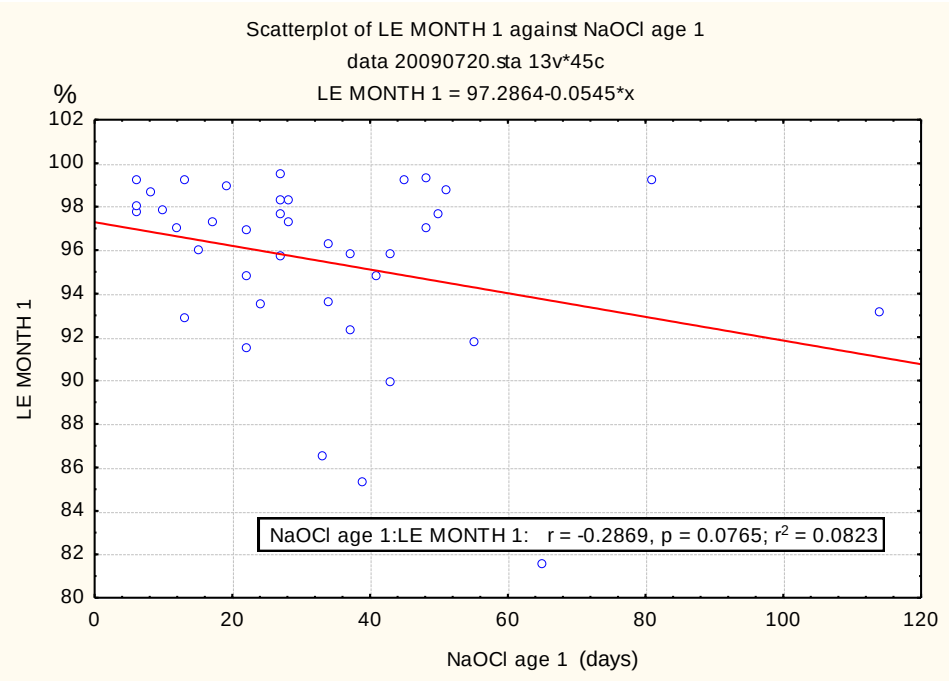

Figure 4.9 B: Scatterplot for Month 1 to show the effect of the age of the NaOCl solution on LE

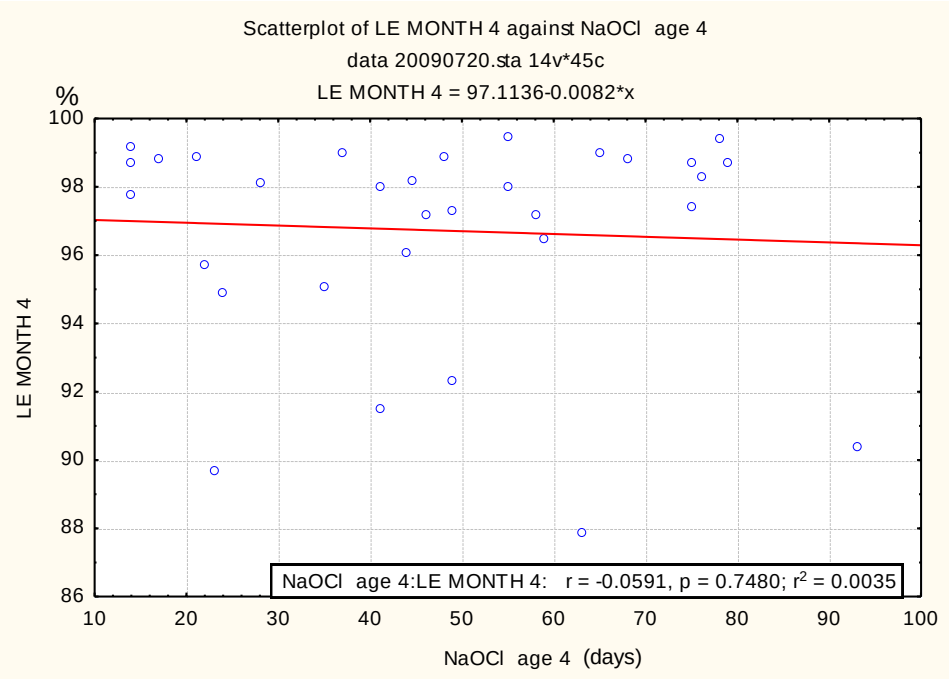

Figure 4.9 C: Scatterplot for Month 4 to show the effect of the age of the NaOCl solution on LE

Summary of results:

- No significant difference between LE in blood samples with and without anti-TB medication could be detected.
- LE varied considerably, between different individuals as well as between samples from the same individual, both in patients and in volunteers.
- The time lapse between sample collection and radiolabelling did not affect LE.
- The age of the reagents used could contribute to variations in LE.

CHAPTER FIVE
DISCUSSION

Tc-99m labelled RBCs are widely used for imaging in nuclear medicine. A significant number of patients being studied with such radiolabelled RBCs are receiving medication that may potentially reduce the labelling efficiency and consequently the imaging result obtained. A range of drugs influences RBC labelling, however there is little documented evidence in controlled studies in large patient populations. An example of a report of the influence of medication on cell labelling is a study performed by Reisdorff et al. (1992:147-150) in which 5 patients were studied to determine the effect of cyclosporin concentration on RBC labelling. Other studies report either *in vitro* or laboratory animal studies (Oliveira et al., 1997:489-494, Oliveira et al., 2000:179-184, Abreu et al, 2006:429-435, Braga et al., 2000:1179-1191). In contrast, the current study included samples from 33 patients on long-term treatment with anti-tuberculosis medication.

During the current study a wide variation in LE was noted throughout the various stages of treatment. An expectation exists that the first phase of treatment would more likely influence the LE, due to a larger number of drugs in more aggressive doses, whereas the second phase of treatment consists of fewer drugs in lower doses. However, no meaningful difference in LE in a specific stage or stages, could be identified.

Neither the Covidien Ultratag package insert, nor textbooks describing RBC labelling with Tc-99m (Owunwanne et al., 1995:70-72, Zolle, 2007:103-108) set a lower limit for labelling efficiency. A labelling efficiency of 90% or more is generally accepted to be suitable for clinical imaging studies. It is important to note that LE's for almost all patient samples in the current study were above 90%. LE's between 80% and 90% occurred more frequently in samples obtained during TB treatment, but as mentioned above, no significant relation between LE and TB treatment could be shown. Other factors which could possibly affect LE will be discussed later in this chapter.

Labelling efficiency reduction due to drug interaction may result from RBC modification such as membrane induced damage resulting in haemolytic anaemia, from competition for the same binding sites, from drug-induced directly or indirectly mediated oxidation of the stannous ion and from a direct inhibition of the stannous and pertechnetate ions by the drug through a chelating effect.

Both isoniazid and rifampicin may induce haemolytic anaemia (Morse, 1989:13-18) (Ammuse & Yunis, 1989:71-82), which could potentially influence RBC labelling. Drug-induced haemolytic anaemia may occur by one of three mechanisms (Salama, 2009:73-79, Johnston, 2009:205-207). Firstly, a drug may bind to the RBC membrane and act as a hapten stimulating production of IgG antibodies, which is the case for isoniazid. Secondly, a drug may first induce the production of IgM antibodies against itself, subsequently the drug-antibody complex binds to the RBC membrane and initiates complement activation, which is the case for rifampicin, resulting in intravascular haemolysis. Finally, a drug may induce the formation of auto-immune anti-erythrocyte IgG antibodies; haemolysis in this setting is typically mild. The current incidence of drug induced haemolytic anaemia is estimated at one per million population, approximately 88% of which results from second and third generation cephalosporins (Patton & Duffull, 1994:445-462). Accordingly, the a priori likelihood for the occurrence of isoniazid and rifampicin drug-induced haemolysis is very low, in keeping with the data presented in this series showing that none of the patients under study presented with haemolytic anaemia. Importantly, with regard to rifampicin, serious side-effects reported with daily therapies as administered in our series mainly consist of hepatotoxicity. Drug induced haemolysis has been especially reported with high dose intermittent rifampicin (Mattson & Jänne, 1982:68-72); none of the patients in our series was on high-dose intermittent rifampicin treatment.

Another cause of drug-induced reduced red blood cell labelling efficiency considered of potential relevance with regard to this thesis was site-binding competition by ethambutol. Ethambutol is a lipophilic drug which diffuses freely into red blood cells. Approximately 1 hour after an intravenous dose, two to three times as much ethambutol is present in the RBC when compared to plasma. To date, the exact binding site of ethambutol in red blood cells has not been identified. The primary binding site of any drug to red blood cells is, however, either associated with haemoglobin (Hb), proteins or with the plasma membrane. As shown by Dewanjee in the early seventies, Tc-99m is mainly bound to the beta chains of HbA1 (Dewanjee, 1974:703-706). Many drugs were shown previously to bind to haemoglobin and to either induce reversible allosteric changes in the haemoglobin molecule or to acetylate haemoglobin, phenomena that might hinder binding of Tc-99m to HbA1. The lack of a significant difference over time in RBC labelling efficiency related to the tuberculosis treatment, including ethambutol, however, suggests that ethambutol does not interfere with the binding of Tc-99m pertechnetate to the haemoglobin beta chain and that ethambutol and its metabolites do not react with the injected Tc-99m pertechnetate or stannous ions.

Red blood cells continuously transport large amounts of oxygen over the course of their approximately 120-day lifespan. This results in a high level of oxidative stress. To counter this level of oxidative stress, red blood cells dispose of an extensive array of antioxidants, including membrane oxidoreductases, cellular antioxidants such as catalase and superoxide dismutase (SOD), and enzymes that continuously produce reducing agents through the glutathione (GSH) system (Kumerova et al., 1998:12-15). Previous studies showed that INH could induce apoptosis in HepG2 cells as well as in other cell lines. This phenomenon was GSH-dependent and antioxidants were shown to have a protective role (Bhadauria et al., 2007:202-214, Chowdury et al., 2006:117-126). The lack of a difference in labelling efficiency between pre- and post-treatment labelled red blood cells suggests that generation of reactive oxygen species (ROS) through INH in red blood cells does not significantly impact labelling with Tc-99m.

Finally, INH was shown to directly complex Tc-99m at room temperature at a physiological pH in the presence of different concentrations of complexing agents and stannous chloride (Yamada et al., 1987:505-511). Thus, it cannot be excluded that part of the Tc-99m complexed by RBC's during our labelling procedure is bound to intra-erythrocyte INH. Similarly, ethambutol may also complex Tc-99m; the chelating effect of ethambutol is due to its chemical structure which is similar to that of penicillamine (Causse et al., 1990:493-496). These effects would increase rather than decrease the LE.

As the current study progressed, questions regarding the reliability of the results arose. Studies on blood from normal volunteers and extra statistical analyses, which were not part of the original study objective, were added to elucidate the influence of possible confounding factors on the results.

When labelling RBC of consecutive sub-samples of a single blood sample of a patient, there will always be a degree of variation in labelling efficiencies. In the normal volunteers there was a variation of 5.2% in one single volunteer and in one of the enrolled patients a variation was noted of 12.3%. This could be due to the amount of supernatant that can be removed, or due to accidental RBC contamination of the supernatant. The procedure is for this very reason, to some extent, operator dependent. To ensure consistency in this respect, all the labellings for this study, were performed by the same person. Other variants that were also considered to play a potential role in the variation in labelling efficiencies of this study, are the time lapsed after each blood sample was taken until labelling started, as well as the age of the sodium hypochlorite solution (NaOCl) and the age of the red blood cell labelling

kit used. Firstly, the time delay between obtaining the blood sample and radiolabelling in the volunteers did not give significant regression at any time interval (20 min, 70 min or 150 min). Similarly, in the study patients, the time delay between obtaining the blood samples and radiolabelling the blood did not have a significant effect on LE.

The second factor that was investigated was the age of NaOCl that was used as an oxidizing agent during the labelling procedure of the study patients. Whilst NaOCl is a cheap and very efficient oxidizing agent it is also chemically unstable. Johnson and Remeikis (Johnson and Remeikis, 1993:40-42) observed that the chemical stability of NaOCl is adversely affected by exposure to high temperature, light and air and by the presence of organic and inorganic contaminants. Exposure to light and air are the two most relevant factors for degradation of NaOCl in the study presented. Time limits (shelf-life) for the use of NaOCl are determined by quality control specifications, such as the concentration of free residual chlorine. Clarkson et al. (2001:269-276) analyzed the decrease in free residual chloride in different sodium hypochlorite solutions stored in conditions similar to actual clinical use. Based on their findings and others, including analytical modelling and the use of the Arrhenius Equation, the reported shelf-life of NaOCl varies widely from 166 days to 690 days, depending on the concentration of the reagent (Nicoletti et al., 2009:27-31). The NaOCl solution (concentration 0.1% w/v) supplied with the Ultratag^{TM} RBC kit has a shelf-life of 15 months (Covidien product information). The NaOCl used in the study was also diluted to a concentration of 0.1% and the recommended shelf-life ranged from four to six months. As the NaOCl used in this study was never more than 6 months old, no marked effect on LE would be expected. However, as evidenced by multiple linear regression analysis the age of the NaOCl used in the study adversely affected RBC labelling efficiency only in the initial drug-free blood samples ($p<0.05$). It should be noted that these were the only blood samples for which NaOCl solution aged 120 to 180 days were used.

The third factor to possibly influence the LE of the sample group is the age of the red blood cell kits used to label the blood samples. The composition of the in-house produced RBC labelling kit for labelling in this study is identical to the commercially available Ultratag^{TM} RBC kit, the shelf-life of which is reported to be 15 months. The in-house prepared RBC kit has a suggested shelf-life of 12 months. The age-related influence of the RBC was also analysed by way of multiple linear regression analysis and it was found that the age of the RBC kit used adversely affected RBC labelling efficiency under baseline conditions (t=0). The most relevant of both factors (age of NaOCl and age of RBC kits) proved to be the age of the RBC-kit (RBC age explained

34.46 % of the variation observed in LE versus 12.86 % for NaOCl). In the results obtained in our study, it was evident that the age of the RBC kits had a larger impact on the LE than the age of the NaOCl. The maximum age of the RBC kits that was used was 358 days, which is close to the limit of the allocated shelf-life. The NaOCl used for labelling had a maximum age of 162 days and therefore far within the prescribed shelf-life. Therefore, the NaOCl had a much lower probability of adversely affecting the LE, than the RBC kits.

The inherent variations in LE are so wide that it would be difficult to detect small changes due to drug effects. The average LE's with intensive phase treatment and without TB medication differed by 1.3%, which may be only due to the above mentioned inherent variations.

Strengths and Weaknesses of this study

Strenghts of this study include the fact that the effect of the medication and metabolites on RBC labelling could be evaluated as opposed to purely *in vitro* studies where blood samples are incubated with drugs and no physiological metabolism takes place.

A previous study was done by *in vitro* incubation of TB-medication with blood samples (Rubow, S, personal communication, 2004). This earlier study, however was only done by incubating drug-free blood samples with different concentrations of TB-medication and therefore the possible influence of the metabolites could not be evaluated. When doing *in vitro* or laboratory animal work, it is often the case that abnormally high or low concentrations of the investigated drug are used such as studies done by Oliveira and co-workers (Oliveira et al., 1997:489-494, Oliveira et al., 2000:179-184, Abreu et al., 2006:429-435, Braga et al., 2000:1179-1191). In the current study, the true concentrations of the TB-medication as used in clinical treatment were used to obtain the results.

Each patient served as its own control, where a baseline labelling was done before commencement of TB-treatment and then the same patient was followed up for the labellings done during the two phases of treatment. This is a better model than comparison of average labelling efficiencies in different groups of patients or volunteers.

Results of RBC labelling are to some extent operator dependant. For instance, one operator may achieve more complete removal of supernatant than another. In the current study, all the labellings throughout the study were performed by the same person in order to limit the possible operator dependant variation.

Many inherent difficulties are encountered in a study involving RBC labelling. Labelling efficiency is influenced by many factors, such as disease, intake of tobacco and dietary habits. These increase the difficulty in proving the influence of a single factor such as patient medication on RBC labelling. The size of the study population and the fact that each patient served as its own control contribute to the reliability of the results and to some extent compensated for the above mentioned influences. Another shortcoming of the study is that the age of reagents was not adequately controlled. As discussed above the age of RBC kits and NaOCl could influence LE.

Conclusion

The aim of this study was to determine if treatment with anti-tuberculosis medication could affect labelling of red blood cells with technetium-99m. Other factors were observed which could possibly influence the results of the study. In order to address these potential causes of misinterpretation of results, a number of factors were then included in the investigation. As discussed above, the RBC labelling technique as such resulted in differences between repeat experiments. The age of the reagents used also influenced the LE. These inherent variations in LE would mask small changes due to drug effects. Patient blood samples during anti-TB treatment met with the arbitrary minimum criterium of 90% LE in most cases. No significant lowering in LE due to the anti-TB medication could be shown.

Neither the first phase of TB treatment (high dose, 4 drug combination) nor the second phase (lower dose, 2 drug combination) of treatment adversely affected red blood cell labelling efficiency when adopting an *in vitro* radiolabelling procedure.

References

Abreu, P.R.C., Almeida, M.C., Bernardo, R.M., Bernardo, L.C., Brito, L.C., Garcia, E.A.C., Fonseca, A.S. and Bernardo-Filho, M. 2006. Guava extract (Psidium guajava) alters the labelling of blood constituents with technetium-99m. *Journal of Zhejiang University SCIENCE B*. 7(6):429-435.

Ammuse, S. and Yunis, A.A. 1989. Drug induced red cell dyscrasias. *Blood Reviews*. 3:71-82.

Bhadauria, S., Singh, G., Sinha, N. and Srivastava, S. 2007. Isoniazid induces oxidative stress, mitochondrial dysfunction and apoptosis in Hep G2 cells. *Cellular and Molecular Biology.* 53(1):202-214.

Braga, A.C., Oliveira, M.B., Feliciano, G.D., Reiniger, I.W., Oliveira, J.F., Silva, C.R. and Bernardo-Filho, M. 2000. The effect of drugs on the labeling of blood elements with technetium-99m. *Current Pharmaceutical Design*. 6:1179-1191.

Brink, H.E. 1959. *Menslike Fisiologie Deel I*. Die Universiteits-uitgewers en Boekhandelaars: 17-27.

Causse, J.E., Pasqualini, R., Cypriani, B., Weil, R., van der Valk, R., Bally, P., Dupuy, A.,Couret, I., Benbarek, M. and Descomps, B. 1990. Labeling of ethambutol with ^{99m}Tc using a new reduction procedure. Pharmacokinetic study in the mouse and rat. *International Journal of Radiation Application and Instrumentation A*, 41(5):493-496.

Chowdury, A., Santra, A., Bhattacharjee, K., Ghatak, S., Saha, D.R. and Dhali, G.K. 2006. Mitochondrial oxidative stress and permeability transition in isoniazid and rifampicin induced liver injury in mice. *Journal of Hepatology*, 45(1):117-126.

Clarkson, R.M., Moule, A.J. and Podlich, H.M. 2001. The shelf-life of sodium hypochlorite irrigating solutions. *Australian Dental Journal*, 46:269-276.

Cole, R.B. 1981. *Monographs in Clinical Pharmacology: Drug Treatment of Respiratory Disease*. London: Churchill Livingstone. 137-150.

Covidien product information: Ultratag^{TM} RBC. *http://imaging.mallinckrodt.com/covidienImaging/pagebuilder.aspx?topicID=132595&page=print* [05/02/2010].

Dewanjee, M.K. 1974. Binding of ^{99m}Tc ion to haemoglobin. *Journal of Nuclear Medicine*.15:703-706.

Dolin, P., Raviglione, M. and Kochi, A. 1994. Global tuberculosis incidence and mortality during 1990-2000. *Bulletin of the World Health Organization*. 72:213-220.

Early, P.J. and Sodee, D.B. 1995. *Principles and Practice of Nuclear Medicine*. New York: Mosby. 109-110.

Fischer, J., Wolf, R. and Leon, A. 1967. Technetium-99m as a label for erythrocytes. *Journal of Nuclear Medicine,* 8:229.

Friedman, L.N. 2001. *Tuberculosis: Current Concepts and Treatment* 2nd Edition. USA: CRC Press LLC.

Gleue, A.D., Spicer, J.A. and Preston, D.F. 1995. Effect of Cyclosporine on Technetium-99m Red blood cell labeling using the Ultratag® RBC kit. *Journal of Nuclear Medicine Technology*. 23(1):22.

Grandjean, L. and Moore, D. 2008. Tuberculosis in the developing world: recent advances in diagnosis with special consideration of the extensively drug-resistant tuberculosis. *Current Opinions in Infectious Diseases.* 21:454-461.

Hambye, A.S., Vandermeiren, A. and Vandevivere, J. 1995. Failure to label red blood cells adequately in daily practice using an in vivo method: methodological and clinical considerations. *European Journal of Nuclear Medicine.* 22:66-68.

Hamilton, R.G. and Alderson, P.O. 1977. A Comparative Evaluation of Techniques for Rapid and Efficient In Vivo labelling of Red Cells with [^{99m}Tc] Pertechnetate. *Journal of Nuclear Medicine.* 18:1008-1011.

Harries, A.D. and Maher, D. 1996: *TB/HIV: A Clinical Manual*. Italy: World Health Organization. 29-30, 85-87.

Hladik, W.B., Nigg, K.K. and Rhodes, B.A. 1982. Drug-Induced changes in the Biologic Distribution of Radiopharmaceuticals. *Seminars in Nuclear Medicine*. 12(2):202.

Houston, S., Pozniak, A. and Ray, C. 1991. Therapeutic Review: Tuberculosis. *Central Africa Journal of Medicine.* 37(8):250-259.

Jeong, Y. and Lee, K. 2008. Pulmonary tuberculosis : up-to-date imaging and management. *American Journal of Roentgenology.* 191:834-844.

Johnson, B.R. and Remeikis, N.A. 1993. Effective shelf-life of prepared sodium hypochlorite solution. *Journal of Endodontics*. Vol 19(1):40-43.

Johnston S.T. 2009. Drug-induced immune haemolytic anemia. Transfus Apher Sci. 205-207.

Kelbaek, H, Gjørup, T. and Fogh, J. 1986. In vivo stability of in vitro labelled ^{99}Tc^{m}-red blood cells for radionuclide determination of left ventricular ejection fractions and volumes. Nuclear Medicine Communications. 7:541-547.

Kirande, S. 2000. Tuberculosis*. South African Health Review*. 336.

Kirande, S. and Bamford, L. 2002. Tuberculosis. *South African Health Review*. 281.

Kumerova, A., Lece, A., Skesters, A., Silova, A. and Petuhovs, V. 1998. Anaemia and antioxidant defence of the red blood cells. *Materia Medica Polona.* 30(1-2):12-15.

Lambert, H.P. and O'Grady, F.W. 1992. *Antibiotic and Chemotherapy Sixth Edition*. London: Churchill Livingstone. 43-47 & 264-267.

Leitl, G.P., Drew, H.M., Kelly, M.E. and Alderson, P.O. 1980. Interference with Tc-99m labeling of red blood cells (RBC's) by RBC antibodies [abstract]. *Journal of Nuclear Medicine*. 21(6): P44.

Le Roux, J.S. 1991. Produksie en kwaliteitskontrole van Tegnesium-99m kitsstelle met spesiale verwysing na 'n kitsstel vir Rooiselmerking*. Master's Thesis, University of Stellenbosch*. 52-53.

Mattson, K. and Jänne, J. 1982. Mild intravascular haemolysis associated with flu-syndrome during intermittent rifampicin treatment. *European Journal of Respiratory Diseases.* 63(1):68-72.

Morse, E.E. 1989. Toxic effects of drugs on erythrocytes. *Annals of Clinical and Laboratory Science*. 18:13-18.

Nicoletti,M.A., Siquiera, E.L., Bombana, A.C. and Oliveira, G.G. 2009. Shelf-life of a 2.5% sodium hypochlorite solution as determined by Arrhenius equation. *Brazilian Dental Journal*. 20(1):27-31.

Oliveira, J.F., Braga, A.C., Avila, A.S., Fonseca, L.M., Gutfilen, B. and Bernardo-Filho, M. 1997. Effect of Thuya occidentalis on the labeling of red blood cells and plasma proteins with technetium-99m. *Yale Journal of Biology and Medicine*. 69(6):489–494.

Oliveira, J.F., Braga, A.C.S., Oliveira, M.B., Avila, A.S., Caldeira-de-Araújo, A., Cardoso, V.N., Bezerra, R.J. and Bernardo-Filho, M. 2000. Assessment of the effect of Maytenus ilicifolia (Espinheira-santa) extracton the labelling of red blood cells and plasma proteins with technetium-99m. *Journal of Ethnopharmacy.* 72:179-184.

Owunwanne, A., Patel, M. and Sadek, S. 1995. The Handbook of Radiopharmaceuticals. England: Chapman and Hall Medical. 70-72.

Pai, M. and O'Brien, R. 2008. New diagnostics for latent and active tuberculosis : state of the art and future prospects. *Seminars in Respiratory and Critical Care Medicine*. 29(5):560-568.

Patton, W.N. and Duffull, S.B. 1994. Idiosyncratic drug-induced haematological abnormalities. Incidence, pathogenesis, management and avoidance. *Drug and Safety.* 11:445-462.

Reisdorff, J.A., Trevino, L.D., Velasques IV, D. and Jackson, M. 1992. The Effect of Cyclosporin Concentration on the Efficiency of in Vitro Technetium-99m Radiolabeling of Red Blood Cells. *Journal of Nuclear Medicine Technology*. 20(3):147-150.

Rubin, E. 2001. *Essential Pathology 3rd Edition*. USA: Lippincott Williams and Wilkins. 316.

Rubow, S.M. 2004. Personal communication.

Ryo, U.Y., Mohammadzadeh, A.A. and Siddiqui, A. 1976. Evaluation of labeling procedures and in vivo stability of Tc-99m red blood cells. *The Journal of Nuclear Medicine*. 17:133-136.

Salama, A. 2009. Drug-induced immune hemolytic anemia. *Expert Opinion on Drug Safety*. 8(1):73-79.

Sampson, C.B. 1990. *Textbook of Radiopharmacy Theory and Practice Volume 3*. New York: Gordon and Breach Publishers,. 210-216.

Sampson, C.B. 1992. Patient medication as a possible cause of unexpected biodistribution of radiopharmaceuticals: a regional survey 1981-1991 and a simple algorithm to predict drug/radiopharmaceutical interaction. *Nuclear Medicine Communications*. 13:229.

Sampson, C.B. 1993. Adverse reactions and Drug interactions with radiopharmaceuticals. *Drug Safety*. 8(3):280-294.

Sampson, C.B. 1996. Complications and difficulties in radiolabelling blood cells: A review. *Nuclear Medicine Communications*. 17:652.

Sampson, C.B. 1998. Interference of patient medication in the radiolabeling of white blood cells: An update. *Nuclear Medicine Communications*. 19:529.

Srivastava, S.C. and Chervu, L.R. 1984. Radionuclide-Labeled Red Blood Cells: Current Status and Future Prospects. *Seminars in Nuclear Medicine*. 14(2):68-82.

Sweetman, S.C., Blake, P.S., McGlashan, J.M. and Neatherco, G.C. eds., 2007. *Martindale: The Complete Drug Reference, 35th edition*. London. Pharmaceutical Press. 245-293.

Tatum, J.L., Burke, T.S., Hirsch, J.I., Miller, W.W. and Fratkin, M.J. 1983. Pitfall to Modified in Vivo Method of Technetium-99m Red Blood Cell Labelling. Iodinated Contrast Media. *Clinical Nuclear Medicine*. 8(12):585-587.

Tortora, G. and Grabowski, S. 1997. *Principles of Anatomy and Physiology 8th Edition*. USA: Harper Collins Publishers. 745-746.

Vidal, M.F., Fonseca, L.M.B. and Bernardo-Filho, M. 1998. Influence of tobacco on the labelling of red blood cells and plasma proteins with technetium-99m. *Journal of Experimental and Clinical Cancer Research.* 17:1-6.

Western Cape Academic Hospitals Antibiotic Recommendations and Wound Care Management 2003. Bayer (Pty) Ltd Healthcare Division. 19.

WHO Fact Sheet. 2008. Epidemiological Fact Sheet on HIV and AIDS. South Africa 2008 Update. WHO. 4

World Health Organization Report. 2009. Global Tuberculosis Control: South Africa 145. *http://www.afro.who.int/en/divisions-a-programmes/atm/tuberculosis/tub-country-profiles.html.* [28 June 2010].

Yamada, N., Takashima, H., Nohara, F., Fujinawa, T., Matsumura, T., Koizumi, K. and Hisada, K. 1987. Investigations of 99mTc labeled tumor seeking agents - usefulness of 99mTc-isoniazid. *Radioisotopes*. 36:505-511.

Zhang, Y., Vilchèze, C. and Jacobs, Jr W.R, 2005. *Tuberculosis and the Tubercle Bacillus*. USA: ASM Press. 118-119.

Zolle, I. 2007. Technetium-99m Pharmaceuticals: Preparation and Quality Control in Nuclear Medicine. Germany: Springer. 103-108.

ADDENDUM A: Details of red cell labelling for all patients

The following table shows:

1. The time delay between the time the blood samples of the study patient were taken and the time that it was labelled in minutes.
2. The age of the RBC kits in days used when labelling the samples.
3. The age of the NaOCl reagent in days used when doing the labellings.
4. The percentage labelling efficiencies (%LE) obtained from each sample.

0 Months (t0/baseline)

SAMPLE		Time delay (min)	RBC Kits (days)	NaOCl (days)	% LE
1	A	54	146	84	98.2
	B	55	146	84	97.2
2	A	59	209	147	95.0
	B	61	209	147	89.0
3	A	47	209	147	97.2
	B	48	209	147	96.6
4	A	100	217	155	91.1
	B	101	217	155	90.6
5	A	40	224	162	89.3
	B	41	224	162	87.7
6	A	36	230	4	99.0
	B	37	230	4	98.7
7	A	43	233	7	95.1
	B	44	233	7	95.3
8	A	40	239	13	97.2
	B	41	239	13	94.2
9	A	35	239	13	95.8
	B	36	239	13	97.6
10	A	51	269	43	93.5
	B	52	269	43	92.7
11	A	31	275	49	97.4
	B	32	275	49	96.3
12	A	Not noted	281	55	98.7
	B	Not noted	281	55	98.8

0 Months continued

SAMPLE		Time delay (min)	RBC Kits (days)	NaOCl (days)	% LE
13	A	44	282	56	96.6
	B	45	282	56	95.0
14	A	51	293	67	99.4
	B	52	293	67	97.6
15	A	43	295	69	99.5
	B	44	295	69	99.0
16	A	39	296	70	95.7
	B	40	296	70	94.0
17	A	98	300	74	95.8
	B	99	300	74	98.2
18	A	40	302	76	94.4
	B	41	302	76	93.0
19	A	36	302	76	92.3
	B	37	302	76	91.1
20	A	38	303	77	96.4
	B	39	303	77	95.3
21	A	42	303	77	98.9
	B	43	303	77	98.9
22	A	34	304	78	97.9
	B	35	304	78	97.7
23	A	40	311	3	98.1
	B	41	311	3	97.6
24	A	50	314	6	99.0
	B	51	314	6	98.3
25	A	110	318	10	99.2
	B	111	318	10	97.2
26	A	40	322	14	98.8
	B	41	322	14	98.6
27	A	57	324	16	99.4
	B	58	324	16	95.6
28	A	34	324	16	97.6
	B	35	324	16	97.3
29	A	40	329	21	93.1
	B	41	329	21	91.6

0 Months continued

SAMPLE		Time delay (min)	RBC Kits (days)	NaOCl (days)	% LE
30	A	44	335	27	97.2
	B	45	335	27	98.2
31	A	52	336	28	92.8
	B	53	336	28	95.0
32	A	43	345	37	95.8
	B	44	345	37	95.3
33	A	60	358	7	95.3
	B	61	358	7	93.8
34	A	74	359	8	96.9
	B	75	359	8	96.1
35	A	78	6	14	99.4
	B	79	6	14	99.3
	C	83	365	14	97.3
36	A	48	12	20	99.0
	B	49	12	20	95.6
	C	88	12	20	97.8
37	A	74	47	55	99.3
	B	75	47	55	98.9
38	A	73	49	57	99.2
	B	74	49	57	98.4
39	A	126	50	58	99.5
	B	127	50	58	99.1
40	A	114	50	58	98.8
	B	115	50	58	98.7
41	A	21	57	65	99.5
	B	22	57	65	99.0
42	A	106	60	78	96.4
	B	107	60	78	95.0
43	A	62	79	105	97.8
	B	62	79	105	97.8
44	A	142	79	13	99.6
	B	142	79	13	99.5
45	A	54	38	44	97.1
	B	55	38	44	96.6

1 Month

SAMPLE		Time delay (min)	RBC Kits (days)	NaOCl (days)	% LE
1	A	40	175	114	93.4
	B	41	175	114	93.0
2	A	12	237	12	95.9
	B	13	237	12	97.3
3	A	18	238	13	92.9
	B	19	238	13	92.9
4	A	17	247	22	95.1
	B	18	247	22	94.6
5	A	23	258	33	85.8
	B	24	258	33	87.1
6	A	32	259	34	94.8
	B	33	259	34	92.4
7	A	28	264	39	84.7
	B	29	264	39	86.0
8	A	43	268	43	91.4
	B	44	268	43	88.4
9	A	50	268	43	97.7
	B	51	268	43	93.8
10	A	40	300	65	81.6
	B	41	300	65	81.6
11	A	41	306	81	99.4
	B	41	306	81	98.9
12	A	47	313	6	99.2
	B	48	313	6	99.2
13	A	20	313	6	97.4
	B	21	313	6	98.1
14	A	26	322	15	96.8
	B	27	322	15	95.2
15	A	33	324	17	97.7
	B	34	324	17	97.0
16	A	21	329	22	92.5
	B	22	329	22	90.5

1 Month continued

SAMPLE		Time delay (min)	RBC Kits (days)	NaOCl (days)	% LE
17	A	44	329	22	96.9
	B	45	329	22	96.9
18	A	49	331	24	94.2
	B	50	331	24	92.7
19	A	70	335	28	97.3
	B	71	335	28	97.3
20	A	32	334	27	98.3
	B	33	334	27	97.1
21	A	31	335	27	98.6
	B	32	335	27	97.9
22	A	13	336	28	98.6
	B	14	336	28	98.1
23	A	43	342	34	95.6
	B	44	342	34	97.0
24	A	30	345	37	92.6
	B	31	345	37	92.1
25	A	48	349	41	95.7
	B	49	349	41	93.8
26	A				
	B				
27	A	37	356	48	97.2
	B	38	356	48	96.7
28	A	48	358	50	97.8
	B	49	358	50	97.6
29	A				
	B				
30	A	39	11	19	98.9
	B	40	11	19	99.0
31	A				
	B				
32	A	29	19	27	95.7
	B	30	19	27	95.6
33	A	30	29	37	96.0
	B	31	29	37	95.5

1 Month continued

SAMPLE		Time delay (min)	RBC Kits (days)	NaOCl (days)	% LE
34	A				
	B				
35	A	61	40	48	99.3
	B	62	40	48	99.4
36	A	19	43	51	99.2
	B	20	43	51	98.5
37	A	38	83	8	99.0
	B	39	83	8	98.8
38	A				
	B				
39	A	66	81	6	99.1
	B	67	81	6	96.9
40	A		88	13	99.3
	B		88	13	99.1
41	A	54	21	27	99.5
	B	55	21	27	99.4
42	A				
	B				
43	A	29	39	45	99.3
	B	30	39	45	99.1
44	A	61	49	55	85.7
	B	62	49	55	98.0
	C	128	49	55	97.3
	D	129	49	55	97.3
45	A	43	79	10	98.1
	B	44	79	10	97.7
	C	45	79	87	96.8

4 Months

SAMPLE		Time delay (min)	RBC KITS (days)	NaOCl (days)	% LE
1	A	23	266	41	98.3
	B	24	266	41	97.7
2	A	42	331	24	95.2
	B	43	331	24	94.6
3	A	14	331	23	89.9
	B	15	331	23	89.4
4	A	45	342	35	95.2
	B	46	342	35	95.0
5	A	188	348	41	91.8
	B	189	348	41	91.2
6	A				
	B				
7	A	44	357	49	92.2
	B	45	357	49	92.4
8	A	104	6	14	99.2
	B	105	6	14	99.1
9	A	41	6	14	98.4
	B	42	6	14	97.2
10	A	38	41	49	97.8
	B	39	41	49	96.8
11	A	60	41	48	98.5
	B	61	41	48	99.3
12	A				
	B				
13	A	54	47	55	99.6
	B	55	47	55	99.4
14	A	85	57	65	99.8
	B	86	57	65	98.2
15	A	36	61	37	99.2
	B	37	61	37	98.7
16	A	60	60	68	98.7
	B	61	60	68	98.8
17	A	112	68	76	98.3
	B	113	68	76	98.3

4 Months continued

SAMPLE		Time delay (min)	RBC KITS (days)	NaOCl (days)	% LE
18	A	57	67	75	97.9
	B	58	67	75	96.9
19	A				
	B				
20	A	105	70	78	99.4
	B	106	70	78	99.4
21	A	71	67	75	98.8
	B	72	67	75	98.7
22	A	47	71	79	99.3
	B	48	71	79	98.1
23	A	66	78	3	97.8
	B	67	78	3	98.5
24	A	94	78	86	97.8
	B	95	78	86	95.5
25	A				
	B				
26	A				
	B				
27	A	52	8	14	98.5
	B	53	8	14	98.8
28	A				
	B				
29	A				
	B				
30	A	90	22	28	98.3
	B	91	22	28	97.9
31	A				
	B				
32	A	82	38	44	96.3
	B	83	38	44	95.9
33	A	63	39	46	97.4
	B	64	39	46	96.9
34	A				
	B				

4 Months continued

SAMPLE		Time delay (min)	RBC KITS (days)	NaOCl (days)	% LE
35	A	57	49	55	97.7
	B	58	49	55	98.2
36	A	11	53	59	95.6
	B	12	53	59	97.4
37	A	55	93	22	96.0
	B	56	93	22	95.3
38	A				
	B				
39	A	54	88	17	98.8
	B	55	88	17	98.7
40	A	58	92	21	98.9
	B	59	92	21	98.9
41	A				
	B				
42	A				
	B				
43	A	31	129	58	97.4
	B	32	129	58	97.0
44	A	53	134	63	87.6
	B	54	134	63	88.3
45	A	61	164	93	94.1
	B	62	164	93	86.8
	C	112	164	93	88.3

ADDENDUM B: Labelling efficiencies at different stages of treatment for all patients

t0 = time 0 (baseline)

1M = 1 month

4M = 4 months

PATIENT NO	t0 (1) %LE	t0 (2) %LE	t0 AVERAGE % LE	1 M (1) %LE	1 M (2) %LE	1 M AVERAGE % LE	4 M (1) %LE	4 M (2) %LE	4 M AVERAGE % LE
1	98.2	97.2	**97.7**	93.4	93.0	**93.2**	98.3	97.7	**98.0**
2	95.0	89.0	**92**	95.9	97.3	**96.6**	95.2	94.6	**94.9**
3	97.2	96.6	**96.9**	92.9	92.9	**92.9**	89.9	89.4	**89.65**
4	91.1	90.6	**90.85**	95.1	94.6	**94.85**	95.2	95	**95.1**
5	89.3	87.7	**88.5**	85.8	87.1	**86.45**	91.8	91.2	**91.5**
6	99.0	98.7	**98.76**	94.8	92.4	**93.6**			
7	95.1	95.3	**95.2**	84.7	86.0	**85.35**	92.2	92.4	**92.3**
8	97.2	94.2	**95.7**	91.4	88.4	**89.9**	99.2	99.1	**99.15**
9	95.8	97.6	**96.7**	97.7	93.8	**95.75**	98.4	97.2	**97.8**
10	93.5	92.7	**93.1**	81.6	81.6	**81.6**	97.8	96.8	**97.3**
11	97.4	96.3	**96.85**	99.4	98.9	**99.15**	98.5	99.3	**98.9**
12	98.7	98.8	**98.75**	99.2	99.2	**99.2**			
13	96.6	95.0	**95.8**	97.4	98.1	**97.75**	99.6	99.4	**99.5**
14	99.4	97.6	**98.5**	96.8	95.2	**96**	99.8	98.2	**99.0**

15	99.5	99.0	**99.25**	97.7	97.0	**97.35**	99.2	98.7	**98.95**
16	95.7	94.0	**94.85**	92.5	90.5	**91.5**	98.7	98.8	**98.75**
17	95.8	98.2	**97.0**	96.9	96.9	**96.9**	98.3	98.3	**98.3**
18	94.4	93.0	**93.7**	94.2	92.7	**93.45**	97.9	96.9	**97.4**
19	93.3	91.1	**92.2**	97.3	97.3	**97.3**			
20	96.4	95.3	**95.85**	98.3	97.1	**97.7**	99.4	99.4	99.4
21	98.9	98.9	**98.9**	98.6	97.9	**98.25**	98.8	98.7	**98.75**
22	97.9	97.7	**97.8**	98.6	98.1	**98.35**	99.3	98.1	**98.7**
23	98.1	97.6	**97.85**	95.6	97.0	**96.3**	97.8	98.5	**98.15**
24	99.0	98.3	**98.65**	92.6	92.1	**92.35**	97.8	95.5	**96.65**
25	99.0 & 99.2	93.9 & 97.2	**97.3**	95.7	93.8	**94.75**			
26	98.8	98.6	**98.7**						
27	99.4	95.6	**97.7**	97.2	96.7	**96.95**	98. 5	98.8	**98.65**
28	97.6	97.3	**97.45**	97.8	97.6	**97.7**			
29	93.1	91.6	**92.35**						
30	97.2	98.2	**97.7**	98.9	99.0	**98.95**	98.3	97.9	**98.1**
31	92.8	95.0	**93.9**						

32	95.8	95.3	**95.55**	95.7	95.6	**95.65**	96.3	95.9	**96.1**
33	95.3	93.8	**94.55**	96.0	95.5	**95.75**	97.4	96.9	**97.15**
34	96.9	96.1	**96.5**						
35	99.4	99.3	**99.35**	99.3	99.4	**99.35**	97.7	98.2	**97.95**
36	99,0	95.6 & 97.8	**97.5**	99.2	98.5	**98.85**	95.6	97.4	**96.5**
37	99.3	98.9	**99.1**	99.0	98.8 & 98.6	**98.8**	96.0	95.3	**95.65**
38	99.2	98.4	**98.8**						
39	99.5	99.1	**99.3**	99.1	96.6	**97.85**	98.8	98.7	**98.75**
40	98.8	98.7	**98.75**	99.3	99.1	**99.2**	98.9	98.9	**98.9**
41	99.5	99.0	**99.26**	99.5	99.4	**99.45**			
42	96.4	95.0	**95.7**						
43	97.8	97.8	**97.8**	99.3	99.1	**99.2**	97.4	97.0	**97.2**
44	99.6	99.5	**99.55**	85.7 & 98.0	97.3 & 97.3	**94.58**	87.6	88.3	**87.95**
45	97.1	96.6	**96.85**	98.1 & 97.7	96.8	**97.53**	94.1 & 86.8	88.3	**89.73**

ADDENDUM C: Results of drug-free volunteers

VOLUNTEER 1

The first volunteer was a female, aged 25

SAMPLE	20 MIN AVE: 96.5% ± 0.9%	70 MIN AVE: 98.2% ± 0.4%	150MIN AVE: 96.3% ± 0.7%
1	97.2%		
2	96.9%		
3	95.5%		
4		98.4%	
5		98.5%	
6		97.7%	
7			97.1%
8			95.9%
9			96.0%

Age of RBC Kits : 20 days
Age of NaOCl : 26 days

VOLUNTEER 2

The second volunteer was a 29 year old male. An identical procedure was performed to volunteer 1.

SAMPLE	**20 MIN** AVE: 96.3% ± 0.4%	**70 MIN** AVE: 97.4% ± 2.0%	**150 MIN** AVE: 96.3% ± 2.4%
1	**96.7%**		
2	**96.2%**		
3	**95.9%**		
4		**99.7%**	
5		**96.4%**	
6		**96.2%**	
7			**99.0%**
8			**95.4%**
9			**94.5%**

Age of RBC Kits : 136days

Age of NaOCl : 65 days

VOLUNTEER 3

The third volunteer was a 25 year old male.

SAMPLE	**20 MIN** AVE: 90.3% ± 0.5%	**70 MIN** AVE: 89.4% ± 0.8%	**150 MIN** AVE: 86.6% ± 0.5%
1	**90.7%**		
2	**90.5%**		
3	**89.7%**		
4		**90.3%**	
5		**89.3%**	
6		**88.7%**	
7			**87.1%**
8			**86.5%**
9			**86.2%**

Age of RBC Kits : 160 days

Age of NaOCl : 89 days

ADDENDUM D: Information sheets and informed consent (English, Afrikaans and Xhosa)

INFORMATION SHEET FOR RED CELL LABELLING STUDY

Dear Sir/Madam

In Nuclear Medicine, several studies require that a patient's blood are labelled with radioactivity.

At Ravensmead Clinic we are currently investigating which factors, such as anti- tuberculosis medication, influence the binding of radioactivity to cells. For this investigation we need volunteers to donate 3 small blood samples of 5 ml (± 1 teaspoon) each. Since you are now starting your treatment with medicine against tuberculosis, you can help us by donating a little bit of your blood. The investigation is purely a laboratory procedure and apart from the blood sample nothing is taken from or administered to the volunteer patient.

Blood samples will be drawn by professionally trained staff (nurses, doctors or radiographers).

A new sterile disposable syringe and needle is used for this purpose as for any other medical examination that needs a blood sample. Withdrawing such a small volume of blood causes no harmful effects. You will receive no extra medication or radioactivity merely for this project.

Blood samples are labeled by code numbers, and not by names, to ensure confidentiality. The results of our study will not influence your treatment at all. We can only study the effect of the medicine on our laboratory radioactivity and can get no other information about you or any illness from your blood.

Your blood sample will be taken to the laboratory in the Nuclear Medicine department, Tygerberg Hospital and treated with radioactivity. As soon as we have finished our measurements, the blood will be destroyed as medical waste.

You may withdraw from this study at any stage without any negative effect. Your TB treatment is not affected by our study.

Your kind co-operation by donating 5 ml blood at 3 different occasions (0 days, 2 months and 4 months during treatment) for our investigation is appreciated very much.

INFORMED CONSENT FOR PARTICIPATION IN RESEARCH PROJECT

FULL NAME OF PATIENT:

…….

CODE NUMBER: …… DATE OF BIRTH: ……

I hereby volunteer to participate in the research project in which the effect of anti-TB medication on the labelling efficiency of red blood cells will be evaluated. I acknowledge that I have been informed about the procedures involved and that I am in receipt of the INFORMATION SHEET TO PATIENTS pertaining to this study.

I understand the contents of the information sheet as well as this form and freely consent to participate.

…… …… ……

PATIENT RESEARCHER WITNESS

SIGNED AT: ……

DATE:…….....

INLIGTINGSBLAD VIR ROOIBLOEDSEL MERKING STUDIE

Geagte Heer/Dame

In kerngeneeskunde is daar verskeie studies wat vereis dat pasiënte se bloed met radioaktiwiteit gemerk word.

Daar word huidiglik ‘n studie by die Ravensmead kliniek gedoen, waar die invloed van anti-tuberkulose medikasie op rooibloedsel merking ondersoek word. Vir dié studie benodig ons vrywilliges om 3 klein bloedmonsters van 5 ml (± 1 teelepel) elk, te skenk. Aangesien u nou met behandeling teen tuberkulose begin, kan u ons help deur ‘n klein bietjie van u bloed te skenk. Die studie word slegs in ‘n laboratoruim gedoen en behalwe die bloedmonster, word niks anders vanaf die vrywillige pasiënt geneem en niks anders aan hom/haar toegedien nie.

Die bloedmonsters word deur professioneel opgeleide personeel getrek (verpleegsters, dokters of radiograwe).

‘n Skoon, steriele, wegdoenbare spuit en naald sal vir die verkryging van die bloedmonsers gebruik word, soos met enige ander mediese ondersoek waar bloed getrek word. Geen skadelike effekte kan veroorsaak word deur so ‘n klein hoeveelheid bloed te skenk nie. U sal geen bykomende medikasie of radioaktiwiteit vir hiedie projek ontvang nie.

Bloedmonsters sal gemerk word met ‘n kode, en nie by naam nie, om sodoende vertroulikheid te verseker. Die uitslae van ons studie sal glad nie u behandeling beïnvloed nie. Ons kan slegs die effek van die medikasie op ons laboratorium radioaktiwiteit bestudeer en kan geen addisionele informasie aangaande u, of enige siektetoestande verkry nie.
U bloedmonster sal geneem word na die laboratorium in die Kerngeneeskunde Departement, by Tygerberg Hospitaal, waar dit met radioaktiwiteit behandel word. Sodra ons die nodige lesings verkry het, sal die bloed vernietig word as mediese afval.

U kan in enige stadium uit die projek onttrek, sonder enige negatiewe effekte. U tuberkulose behandeling sal nie geaffekteer word deur ons studie nie.

U vriendelike samewerking deur 5 ml bloed by 3 geleenthede (0 dae, 2 maande en 4 maande gedurende behandeling) te skenk, sal hoogs waardeer word.

INGELIGTE TOESTEMMING VIR DEELNAME AAN NAVORSINGS PROJEK

VOLLE NAAM VAN PASIËNT:

…….

KODE NOMMER: …… GEBOORTE DATUM: ……

Hiermee is ek gewillig om deel te neem aan die navorsings projek, waar die effek van anti-tuberkulose medikadie op rooibloedsel merking geëvalueer word. Ek verklaar dat ek ingelig was aangaande die betrokke prosedures en dat ek in besit is van die inligtings blad vir rooibloedsel merking.

Ek verstaan die inhoud van die inligtings blad sowel as hierdie vorm en ek stem vrylik in om deel te neem.

…… …… ……

PASIËNT NAVORSER GETUIE

GETEKEN BY:……

DATUM:……

IPHEPHA NGOWAZI NGEZIUNDO NGESELI EZIBOMVU (“Red Cell”)

Mhlekazi obekekileyo

Kumayeza eNuclear, kufunyaniswe ukuba kwizifundo ezininzi igazi lezigulana zileyibhelishwe ngeradiyo ektivithi.

KwiKliniki yase Ravensmead senza uphando lokokuba zeziphi na iimeko, ezinje ngamayeza alwa nesifo sephepha ezichaphazela ukubamba iradiyo ektivithi kwiseli (cells). Koluphando kufunwa amavolontiya okunikezela ngesampule zegazi ezintathu ezimalunga netisipuni kuphela. Njengokuba uqala ukusebenzisa amayeza ukunqanda lentsholongwane yesifo sephepha, thina ingasinceda ngokuthi uphise ngentwana encinci yegazi lakho. Olu phando lwenziwa elabholetri kwaye akukho nto ithathwayo nefakwayo kwezizigulana zinikezela ngegazi. Olu tsalo gazi lwenziwa zingcaphephe, abantu abaqeqeshelwe oko abafana namanesi, oogqirha kunye neradiyografazi (radiographers).

Kusetyenziswa iinaliti nezarinji ezintsha, xa kusenziwa uvavanyo lotsalo gazi. Yintwana nje yegazi ethatyathwayo kwaye ayinabungozi kule projekthi awufumani mayeza wambi kunye neradiyo ektivithi engenye. Ukuqinisekisa imfihlakalo eyenzelwa phantsi kwalo oluphando isampuli zegazi zileyibhelishwa ngokwenombolo yekhodi hayi ngamagama. Iziphumo zesifundo eziluchaphazeli unyango, kwaye into efunwayo lufundo ngendlela amayeza asebenza ngayo ngesigulana okanye isigulo esilapho egazini.

Ezi sampuli zegazi zisiwa elabhoretri ekwicandelo le “Nuclear Medicine” kwisibhedlele sase Tygerberg, lize linyangwe nge radiyo ektivithi. Emveni kokuba lisetyenzisiwe igazi elo, liye litshatyalaliswe. Izifundo zethu aziluchaphazeli unyango lwakho lwesifo sephepha. Siyabuvuyela ubuntu bakho bokunikezela nge 5 ml yegazi amatyeli amathathu (imini, inyanga ezimbini kunye neenyanga ezintandathu ngexa lo nyango) koluphando lwesifundo sethu.

IMYUME YOKUTHABATHA INXAXHEBA KUPHANDO LWEPROJEKTHI

IGAMA LESIGULANA:……..

INOMBOLO YEKHODI:…….....

UMHLA WOKUZALWA:……..

Ndifuna ukuthabatha inxaxheba kuphando lwaleprojekthi apho kuvavanywa inxaxheba ethatyathwa ngamayeza okuthintela isifo sephepha (T.B.). Ndiyavuma ukuba ndixelelwe ngekqubo kwaye ndinalo iphepha lolwazi ngezigulana ngokubhekiselele kwesisifundo. Ndiyasiqonda isiqulatho sephepha lolwazi, ngoko ke ndiyavuma ukuthabatha inxaxheba.

…….. …….. …...…….

ISIGULANA UMPHANDI UGQIRHA

ISAYINWE E:……..

UMHLA: ……